Knee osteoarthritis, its comorbidity and the role of vitamin D

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(Medical Research)

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DECLARATION OF ORIGINALITY

I declare this thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information duly acknowledged in the thesis, and to the best of my knowledge and belief, no material previously published or written by any other person, except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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STATEMENT OF ETHICAL CONDUCT

The research associated with this thesis abides by the International and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

The Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616) approved this project, Vitamin D Effect on Osteoarthritis (VIDEO) study. All participants wrote informed consent.

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Chapter 5

Zheng S, Wang B, Han W, Zhu Z, Wang X, Jin X, Antony B, Cicuttini F, Wluka A, Winzenberg T, Aitken D, Blizzard L, Jones G, Ding C. Vitamin D supplementation and inflammatory and metabolic biomarkers in patients with knee osteoarthritis: post hoc analysis of a randomised controlled trial. *British Journal of Nutrition* 2018; 120(1):41-48.

Chapter 6

Zheng S, Tu L, Cicuttini F, Han W, Zhu Z, Antony B, Wluka A, Winzenberg T, Meng T, Aitken D, Blizzard L, Jones G, Ding C. Effect of vitamin D supplementation on depressive symptoms in patients with knee osteoarthritis. *Journal of American Medical Directors Association* 2018; pii: S1525-8610(18)30497-3.

OTHER PUBLICATIONS

Zheng S, Xu J, Xu S, Zhang M, Huang S, He F, Yang X, Xiao H, Zhang H, Ding C. Association between circulating adipokines, radiographic changes, and knee cartilage volume in patients with knee osteoarthritis. *Scandinavian Journal of Rheumatology* 2016;45(3):224-229.

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Wu J, Xu J, Wang K, Zhu Q, Cai J, Ren J, Zheng S, Ding, C. Associations between circulating adipokines and bone mineral density in patients with knee osteoarthritis: A cross-sectional study. *BMC Musculoskeletal Disorders* 2018;19(1).

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SCIENTIFIC PRESENTATION AND AWARDS ARISING

FROM THE THESIS

2016

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2017

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"Vitamin D supplementation and biomarkers of inflammation in patients with knee osteoarthritis: post-hoc analysis of a randomised controlled trial" ***Poster Presentation***

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"Factors associated with depression and effects of vitamin D supplementation and maintaining vitamin D sufficiency on depression in patients with knee osteoarthritis" ***Poster Presentation***

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"Vitamin D supplementation improves depression in knee osteoarthritis patients over 24 months" ***Poster Tour Presentation***

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ABSTRACT

Osteoarthritis (OA) is the most common chronic joint disease. Although disease progression is usually slow, it can ultimately lead to joint failure with pain, stiffness, swelling and disability. Additionally, patients with OA are at significantly higher risk of developing comorbidity than without OA. Coexisting with comorbidity results in increased difficulties in OA management, reduced quality of life and increased disease burden. Up to date of this thesis, OA disease-modifying therapy remains the greatest unmet. Hence, there is an urgency for developing an effective treatment to slow the disease progression and identifying modifiable risk factors for OA comorbidity.

This thesis aims to use a mixed approach to investigate multiple aspects of OA, including the effect of vitamin D on the disease and its comorbidity and factors associated with its comorbidity. It is based on the Vitamin D Effects on Osteoarthritis (VIDEO) study, which is a randomised clinical trial and aims to examine the effects of monthly vitamin D supplementation over two years on knee pain and knee structural changes in symptomatic knee OA patients with low vitamin D levels. 413 participants (mean age: 63.2 years; 50% female) with symptomatic knee OA and low 25(OH)D levels (>12.5nmol/L and <60nmol/L) were randomised to treatment and control groups in Hobart and Melbourne.

Chapter 4 describes the differences in disease progression and symptoms among people with knee OA by vitamin D status over time, which is a post hoc analysis of the original VIDEO study. Participants who maintained vitamin D sufficiency over two years had decreased loss of

tibial cartilage volume, less increase in effusion-synovitis volume and more improvement in knee joint physical function compared with those who did not. This suggests beneficial effects of maintaining vitamin D sufficiency on cartilage loss, effusion-synovitis and physical function in patients with symptomatic knee OA.

Chapter 5 investigates whether vitamin D supplementation and maintaining vitamin D sufficiency are associated with changes in inflammatory and metabolic biomarkers over two years in patients with knee OA and vitamin D deficiency. Vitamin D supplementation had no significant effects on changes in serum hs-CRP, IL-6, IL-8, IL-10, leptin, adiponectin, resistin, adipsin and apelin, compared to placebo. Furthermore, being consistently vitamin D sufficient over two years was also not associated with changes in these biomarkers compared to not being consistently sufficient. These do not suggest vitamin D supplementation and maintaining vitamin D sufficiency may alter systemic inflammation in knee OA patients.

Chapter 6 investigates the effect of vitamin D supplementation and maintaining vitamin D sufficiency on depressive symptoms in patients with knee OA patients. Depressive symptoms improved more in the vitamin D treatment group and the participants who maintained vitamin D sufficiency between month 3 and 24 over 24 months, compared to the placebo group and the group which did not maintain sufficient vitamin D, respectively. Although the improvement was small, and the clinical importance was uncertain, vitamin D supplementation and maintaining vitamin D sufficiency could reduce depressive symptoms in patients with knee OA. These suggest that vitamin D supplementation and maintaining adequate vitamin D levels over 24 months may be beneficial for depressive symptoms in patients with knee OA.

Chapter 7 describes the temporal relationships between demographic and OA clinical factors, joint symptoms and depression in patients with symptomatic knee OA. The prevalence and the incidence of depression was 25.4% and 11.2%, respectively. The common OA risk factors such as higher BMI, lower education level and having two or more comorbidities were associated with prevalent depression and being female was associated with incident depression in knee OA patients. Also, higher levels of knee pain and physical dysfunction and having multi-site pain were associated with increased risks of both prevalent and incident depression. These findings provide empirical evidence that management of common OA risk factors, chronic pain and joint dysfunction may be beneficial for preventing and managing depression in knee OA patients.

Chapter 8 investigates the effect of vitamin D supplementation and maintaining vitamin D sufficiency on foot pain in patients with knee OA patients. In this sample, 51.8% of participants reported they had foot pain in patients with knee OA and vitamin D deficiency. Foot pain improved more in the vitamin D treatment group and the participants who maintained vitamin D sufficiency between month 3 and 24 over 24 months, compared to the placebo group and the group which did not maintain sufficient vitamin D, respectively. These suggest that vitamin D supplementation and maintaining adequate vitamin D levels over 24 months had beneficial effects on foot pain deterioration in OA patients.

In summary, this thesis indicates that depression and foot pain are common comorbidities in patients with knee OA. Management of common OA risk factors, chronic pain and joint dysfunction may be beneficial for the prevention and management of depression in knee OA patients. Maintaining vitamin D sufficiency may be beneficial on cartilage loss, effusion-synovitis and physical function in people with symptomatic knee OA. Vitamin D

supplementation and maintaining vitamin D sufficiency may not affect systemic inflammation but may have beneficial effects on comorbidities including depression and foot pain in patients with knee OA.

LIST OF ABBREVIATIONS

$1,25(OH)_2D$	1,25-hydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
3D	3-Dimension
95% CI	95% Confidence Interval
ABS	Australia Bureau of Statistic
ACR	American College of Rheumatology
BMLs	Bone Marrow Lesions
BMD	Bone Mineral Density
CGRP	Calcitonin Gene-Related Peptides
CV	Coefficient of Variation
DALY	Disability-Adjusted Life Years
ECM	Extracellular Matrix
EULAR	European League Against Rheumatism
GBD	Global Burden of Disease
ICC	Intra-class Correlation Coefficient
IPAQ	International Physical Activity Questionnaire

JSN	Joint Space Narrowing
K&L	Kellgren and Lawrence
MCID	Minimal Clinically Important Difference
MFPDI	Manchester Foot Pain and Disability Index
MMPs	Matrix Metalloproteinases
MRI	Magnetic Resonance Imaging
NHS	National Health Survey
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
OR	Odds Ratio
PHQ-9	Patient Health Questionnaire
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RCT	Randomized Controlled Trial
ROA	Radiographic Osteoarthritis
ROI	Region of Interest
SD	Standard Deviation
TJR	Total Joint Replacement Surgery

US	United States
VAS	Visual Analogue Scale
VDR	Vitamin D Receptor
VIDEO	Vitamin D Effect on Osteoarthritis
WHO	World Health Organization
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
WORMS	Whole-Organ Magnetic Resonance Imaging Score
YLD	Years Lived with Disability

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Chapter 1 Introduction

1.1 Overview of osteoarthritis

Osteoarthritis (OA) is the most prevalent chronic joint disease and a major cause of joint pain, disability and reduced quality of life in elderly people[1]. In past few decades, the definition of OA has been changed from the degenerative disease to a progressive disease[2]. The Osteoarthritis Research Society International (OARSI) has defined OA as "a disorder involving movable joints characterised by cell stress and extracellular matrix degradation initiated by micro and macro injuries that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity"[3]. The most common risk factors, including systemic and local risk factors, are age, female sex, genetics, diet, metabolic syndrome, obesity, occupation and injury[1]. OA is not merely a process of wear and tear, but rather, an abnormal remodelling of whole joint structures, which are characterised by cartilage degradation, bone remodelling, osteophytes formation, variable degrees of inflammation of the synovium, and degeneration of ligaments[4]. Furthermore, there can also be changed in periarticular muscles, nerves, joint capsule, and local pad that may contribute to OA or the symptoms of OA[4].

It has been recognised that OA may develop from initial biochemical change in any of joint tissue to the presence of symptoms[2 5]. Although disease progression is usually slow, it can ultimately lead to joint failure with pain, stiffness, swelling and disability in a large proportion patients[6]. Joint pain is the primary clinical symptom in OA and a key determinant for seeking medical care. Current guidelines have addressed to relief the joint symptoms, particular in improved function and pain relieving[7]. However, there is no effective treatment existing to slow the structural progression. In the severe stage of disease progression, most patients are seeking for joint replacement surgery. In addition, people with OA often have other chronic

comorbidities[8]. Moreover, the presence of comorbidities in older adults with OA is associated with more pain, greater dysfunction, and worse prognosis and management of OA and contributed to substantial burdens[9-11]. Therefore, there is an urgency for developing effective treatments to slow the disease progression and comorbidity.

1.1.1 Epidemiology of OA

1.1.1.1 Definition

OA definition can influence prevalence and incidence estimates[12]. For the epidemiologic investigation, OA is defined as radiographic OA (ROA), clinical OA and symptomatic OA. There are several radiographic scoring systems existing. Of these, the Kellgren and Lawrence (K&L) grade system is the most widely used to identify and assess the grade of OA[13]. The overall grades of K&L joint scoring system range from 0 to 4, and are related to osteophytes and joint space narrowing (JSN), as follows[14]:

grade 0, none: no features of OA;

grade 1, doubtful: questionable osteophytes or questionable JSN;

grade 2, minimal: definitive small osteophytes, little or mild JSN;

grade 3, moderate: definitive moderate osteophytes, JSN of at least 50%;

grade 4, severe: joint space impaired severely, cysts and sclerosis of subchondral bone.

The grade greater than 2 with definitive osteophyte or JSN is defined as the presence of OA[15]. The World Health Organization (WHO) adopted these criteria as the standard for epidemiological studies on OA. However, there is a discordance between the radiographic severity and symptoms; individuals with early painful OA may be free from radiographic changes and, vice versa[16]. Symptomatic and clinical OA criteria have been developed by a subcommittee of the American College of Rheumatology (ACR)[17-19]. It is generally defined by the presence of symptoms in a joint with OA, which is more clinically relevant compared to the OA. The definition of symptomatic OA is combining radiological and clinical features, which is the most important in public health[20].

1.1.1.2 Prevalence

Data from the global burden of disease (GBD) 2013 study has shown that OA affects nearly 241 million people globally[21]. In addition, OA may develop in any joint, but most commonly affects the hands, hips and knees[22]. The prevalence of OA varies according to the definition of OA, the specific joint(s) under study, and the characteristics of the study population[23]. According to the GBD 2010 study, knee OA is a more common condition than hip OA with the prevalence of 3.8% (radiographically confirmed symptomatic knee OA) and 0.85% (radiographically confirmed symptomatic hip OA), respectively[24]. The prevalence increased consistently with age and was higher in female than male (Figure 1.1).

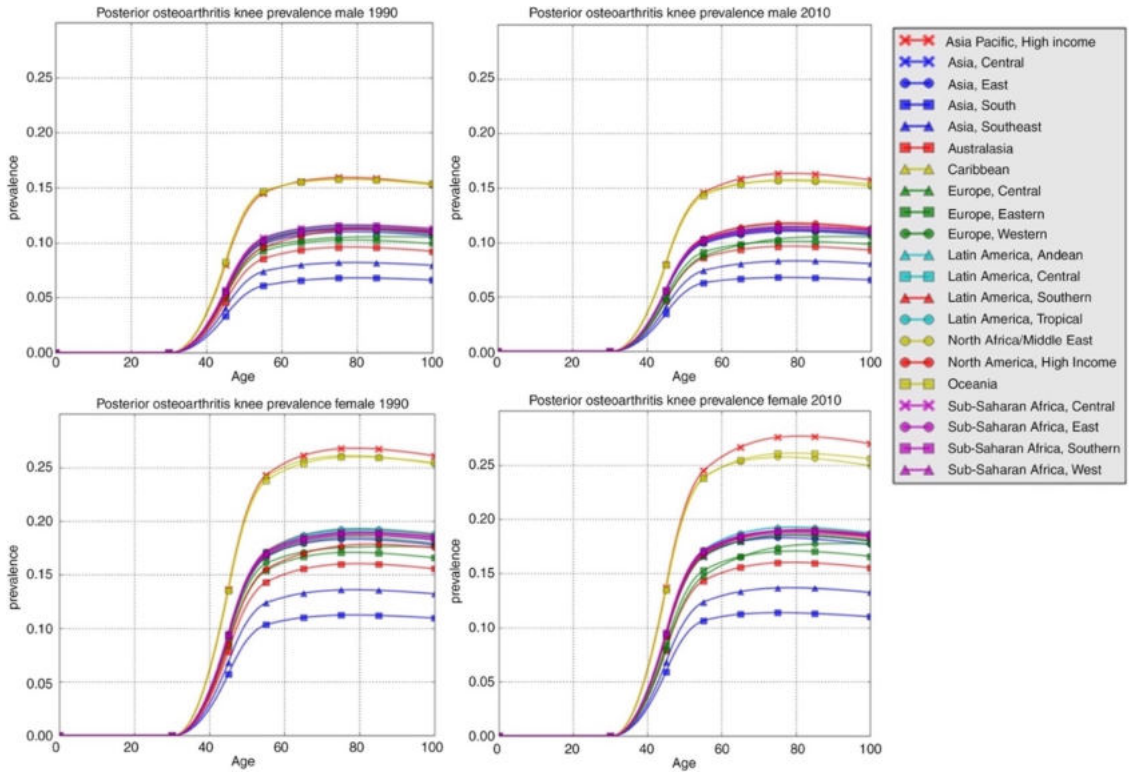


Figure 1.1 Prevalence of knee osteoarthritis by age, sex, year and region, GBD 2010 study in 1990 and 2010

Source: Cross M, et al. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014;73(7):1323-30.

Prevalence of knee OA was highest in the Asia Pacific high-income region, and the prevalence of hip OA was highest in the North America high-income region in 2010 (Figure 1.1). In Australia, approximately 2.1 million (9%) Australians report suffering OA, which is over half (59%) of all arthritic conditions, according to the National Health Survey (NHS) in 2014–15[25]. The prevalence increases sharply from the age of 45 years, and 1 in 5 Australians (21%) over the age of 45 have OA. Moreover, there has been a slight increase in the age-standardised prevalence of OA, from 7.5% in 2001 to 8.1% in 2014–15 (Figure 1.2)[25]. With increasing risk factors for OA, the prevalence of OA is increasing, and this increasing prevalence of OA is expected to continue. The global prevalence of hip and knee OA is projected to increase as the population ages.

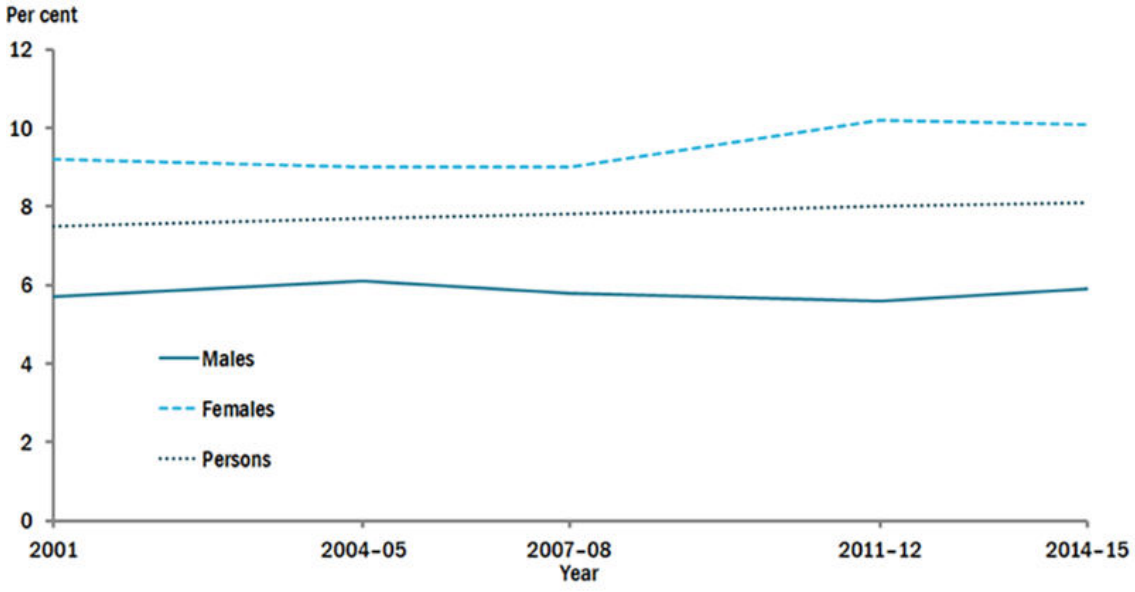


Figure 1.2 Prevalence rate of OA, by sex, 2001 to 2014–15 in the Australian population

Source: Australia Bureau of Statistic (ABS). National Health Survey: First Results, 2014–15. 2015.

1.1.1.3 Risk factors

OA has a multi-factorial aetiology, with different sets of risk factor[26]. The WHO defines risk factor as any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease. The risk factors of OA have been broadly divided into person-level factors and joint-level factors. The developing of OA appears to be the results of the complex

interplay between person-level factors and joint-level factors in any given individual[23]. The person-level factors include increasing age, female sex, obesity, genetics, race/ethnicity and nutritional factors, which may represent genetic or sociocultural influences and result in susceptible individuals. The joint-level factors include occupation, physical activity, injury, muscle strength, alignment, leg length inequality and bone/joint morphology, which are reflective of mechanisms related to abnormal loading of the joints and result in susceptible joint[26].

When considering non-modifiable factors for OA, age and sex are the strongest predictors. OA is not a simple consequence of joint aging and repeated "wear and tear", but a consequence of cumulative exposure to various risk factors and biologic changes that occur with aging, such as chondrocyte senescence, loss of cartilage matrix and oxidative damage[27]. Furthermore, the basic cellular mechanisms that maintain tissue homeostasis decline with age, leading to an inadequate response to stress or joint injury and resultant joint tissue destruction and loss. Strong epidemiologic evidence links female gender to an increased risk of OA. Females having higher fat mass, lower muscle mass and weaker muscle may explain some of the gender difference in OA susceptibility. The sex endogenous hormonal factors and reproductive factors may also play key roles in the pathogenesis of OA with complex mechanisms, especially in menopause females[28].

In contrast, obesity and vitamin D deficiency are modifiable risk factors. Obesity is associated with increased rates of OA. For every 11 pounds of weight gain, there is a 36 per cent increased risk for developing OA[29]. The increased prevalence was attributed to biomechanical factors and low-level inflammation. Increasing epidemiological evidence has suggested that insufficient serum vitamin D status is associated with the progression of OA and worsening in its symptoms[30]. Most modifiable risk factors arise primarily because of unhealthy diets or lifestyle choices. Evidence has shown management of modifiable risk factors could be a key intervention to improve the OA. For instance, weight loss and exercise are beneficial to reduce pain, improve function and inhibit inflammation[31]. Hence, recognition of the crucial role of modifiable risk factor should be a priority in developing OA treatments[32]. In the following sections, we will describe vitamin D deficiency as a modifiable risk factor for OA disease onset and progression.

1.1.1.4 Disease burden

OA is associated with decreased physical activity and joint instability, and is one of the leading causes of disability worldwide. Globally, hip and knee OA was raised as the 13th highest contributor to the global disability (years lived with disability, YLD) in the GBD 2013 study and 26th highest in the disability-adjusted life years (DALYs) in the GBD 2017 study[21 33]. As a consequence of that the GBD data only include hip and knee OA, the real global burden of OA is likely to be underestimated. The mean YLDs increased by 75% from 1990 to 2013, which was reported to be the 4th fastest increasing condition. With the aging of the population and the increasing rate of obesity throughout the world, the considerable burden of OA continues to increase, particularly in the developed country.

OA is also associated with a particularly high economic burden, largely attributed to the medical costs and loss of productivity in direct and indirect cost. In the United States (US), OA was the second most costly health condition treated in 2013. In that year, the total national arthritis-attributable medical care costs and earning losses among adults with arthritis were $303.5 billion, $139.8 billion for medical expenditures and $163.7 billion for earning loss[34]. In Australia, there were $1.6 billion costs in OA management in the year 2008 to 2009. It was accounted for 29% of health-care expenditure on arthritis and other musculoskeletal conditions, and consisted of 77% admitted patients cost, 17% out-of-hospital cost and 6% prescription medication[35]. Therefore, the impact of OA on individuals and the health system is significant. Greater attention to prevention and slowing the disease progression are required.

1.1.2 Disease progression and monitoring

OA disease progression is typically defined as increasing structural disease severity[36]. Early identification of OA is most important to improve clinical decision-making and advance the understanding of disease progression and treatment options. The conventional plain radiographs are the most common and accessible tool to detect bony features associated with OA, including osteophytes, subchondral bone sclerosis and cysts[18]. The K&L grade, based on the radiographic imaging, is a widely accepted semi-quantitative scoring system to assess the disease stage of OA. However, the conventional radiograph imaging technique is not a sensitive measure of changes and weakly associated with symptoms[37 38]. For example, in the

early stage of radiographic changes that are presented on radiography, there is already 10% cartilage loss, and over 40% of patients have had cartilage defects (Figure 1.3)[39-41].

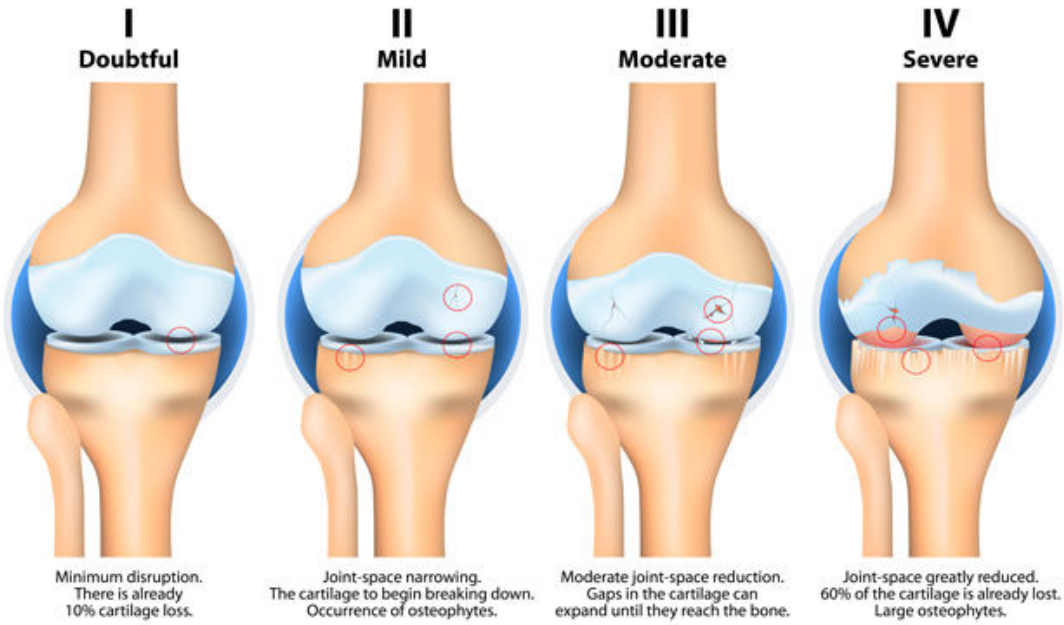


Figure 1.3 Disease progression of the knee OA

Source: https://thumbs.dreamstime.com/z/stages-knee-osteoarthritis-oa-kellgren-lawrence-criteria-assessment-stage-classifications-45644007.jpg

Owing to the remarkable development of imaging technique, the magnetic resonance imaging (MRI) is a superior and more sensitive technique than plain radiography, which allows examining the whole joint structural changes, especially in the early stage (Table 1.1)[42]. Strengths of MRI include its ability to visualise individual tissue pathologies that are not detected on radiography, as well as the interrelationship between tissue pathologies[43]. MRI assessments are mainly classified into quantitative morphometry and semiquantitative scoring systems, for instance, quantitative MRI measures of cartilage morphology and the Whole-Organ Magnetic Resonance Imaging Score (WORMS) to assess typical OA structural change of cartilage defects, bone marrow lesions (BMLs) and effusion-synovitis[44].

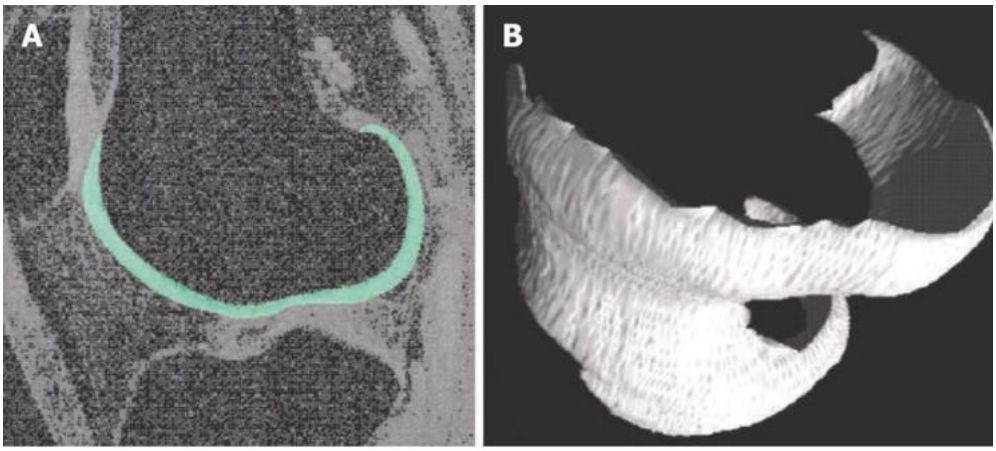
Table 1.1 Different disease stages of OA

Stage		Changes
I.	Initial stage	Compositional changes: – Biochemical changes reflecting break down of cartilage matrix proteins – MRI-detected compositional changes reflecting increased water content or damage of cartilage matrix proteins
II.	Earlier to middle stage	MRI-evident changes: – Bone expansion, attrition, and bone marrow lesions – Cartilage defects and cartilage loss – Meniscal tear and ligament tears – Synovitis – Periarticular cysts and bursitis
III.	Joint failure stage	Radiographically evident changes: – JSN – Osteophytes, subchondral sclerosis, and cysts
IV.	Joint death stage	Joint replacement

Source: Ding CH, et al. Use of imaging techniques to predict progression in osteoarthritis. Curr Opin Rheumatol 2013;25(1):127-35.

Cartilage volume loss is the hallmark of OA. Cartilage volume as assessed by MRI is a reproducibly quantitative and accurate measurement (Figure 1.4)[45]. The loss of cartilage volume is associated with radiographic progression, knee pain and change in pain over times[39] [46-48]. MRI assessment of cartilage morphology is now recommended for the assessment of disease progression as an endpoint for clinical trials[49] [50]. Cartilage defects are localized lesions or tears within the cartilage, which can be visualized directly from MRI (Figure 1.5)[51]. It was noted that cartilage defect may not be the same process with cartilage volume loss. It is an important marker of early cartilage damage and may reflect a poor capacity for cartilage repair or trauma. Although the scoring of cartilage defects is a semi-quantitative and cannot be accurate as cartilage volume loss, the progression of cartilage defects can be regarded as an increasing split in cartilage rather than cartilage volume loss[40].

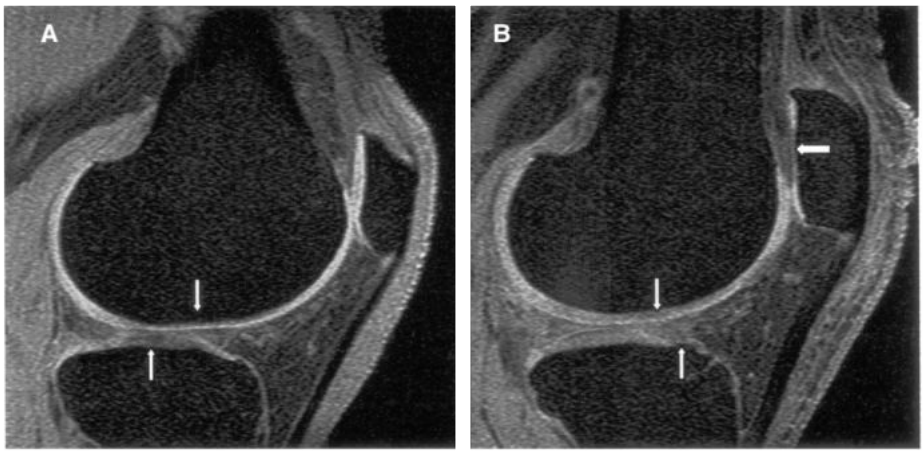
Figure 1.4 Quantitative 3-dimension (3D) analysis of cartilage morphology from MRI



A: Sagittal MRI of human knee obtained with a fat-suppressed sequence, femoral cartilage is segmented; **B:** 3D volume reconstruction of the femoral cartilage.

Source: Wang YXJ,et al. Non-invasive MRI assessment of the articular cartilage in clinical studies and experimental settings. World Journal of Radiology *2010;2(1).*

Figure 1.5 Representative sagittal T1-weighted fat-suppressed 3D MRI illustrating cartilage defects grades



A: Normal patellar cartilage but with cartilage defect of grade 1 at tibial (up arrow) and femoral (down arrow) sites; **B:** Cartilage defects of grade 2 at tibial site (up arrow), of grade 1 at femoral site (down arrow), and of grade 3 at patellar site (left arrow).

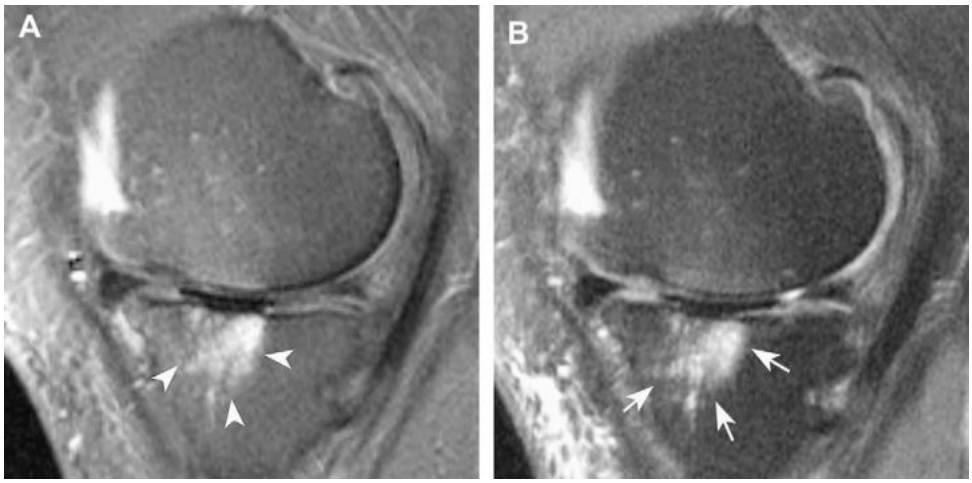
Source: Cicuttini F, et al. Association of cartilage defects with loss of knee cartilage in healthy, middle-age adults: A prospective study. Arthritis & Rheumatism *2005;52(7):2033-39.*

BMLs, shown on MRI, is described as regions of an ill-defined area of signal alteration (Figure 1.6)[52]. BMLs reflect a wide range of abnormalities such as abnormal trabeculae, bone marrow necrosis/remodelling, contusion and marrow fibrosis. A number of studies have demonstrated the BMLs could predict the disease progression of OA[53-55]. Effusion and/or synovitis are the hallmarks of joint inflammation and can be measured using MRI quantitatively and semi-quantitatively[56 57]. The effusion-synovitis measured on MRI are inflamed synovium and synovial fluid[58]. It could occur in early-stage and late-stage of OA and is also associated with structural and symptomatic progression, as well as an increased risk of developing OA[59-61].

Therefore, cartilage volume loss, cartilage defects, BML and effusion-synovitis could be used to monitor the OA disease progression[5]. Furthermore, these structural changes are predictors

of joint replacements[41 62 63]. MRI has become a valuable tool to examine the multiple tissues abnormalities and assess the disease progression in OA patients.

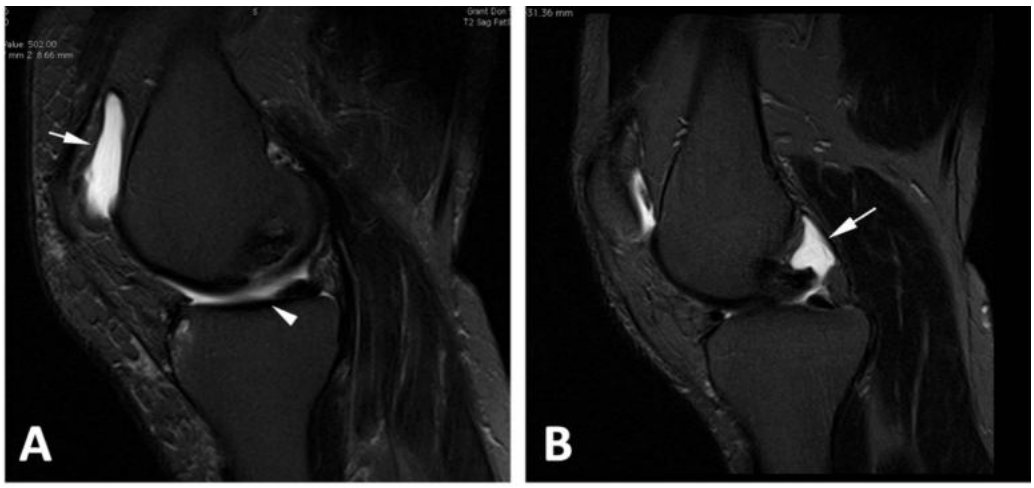
Figure 1.6 Subchondral BMLs in OA on T1-weighted fat-suppressed image



A: Tibial BML is depicted as diffuse hyperintensity (arrows); **B:** After intravenous gadolinium administration, BMLs were shown with similar extent (arrowheads).

Source: Roemer FW, et al. MRI-detected subchondral bone marrow signal alterations of the knee joint: terminology, imaging appearance, relevance and radiological differential diagnosis. Osteoarthritis and Cartilage 2009;17(9):1115-31.

Figure 1.7 T2-weighted fat-saturation fast spin echo sagittal images for effusion-synovitis in different subregions



A: Grade 3 effusion-synovitis in the suprapatellar pouch (arrow) and grade 1 effusion-synovitis in the central portion (arrowhead); **B:** Grade 3 effusion-synovitis around posterior cruciate ligament (arrow).

Source: Wang X, et al. Association between MRI-detected knee joint regional effusion-synovitis and structural changes in older adults: a cohort study. Annals of the Rheumatic Diseases 2016;75(3):519.

1.1.3 Management

Joint pain is the primary clinical symptoms in OA and a key determinant for seeking medical care. Due to the incurable condition of OA, in the past few years, international evidence-based guidelines, particularly the newly released European League Against Rheumatism (EULAR) recommendation, have addressed to manage symptoms, in particular controlling pain and improving function and quality of life[64-66]. There is a broad spectrum of interventions is available including nonpharmacological approaches, pharmacological treatments and surgery. Optimally, patients are receiving a combination of nonpharmacological and pharmacological therapies. However, there is a gap between evidence and practice[67 68].

The nonpharmacological therapies comprise patient education, exercise, orthotics and weight management, which have shown the promising effect of symptoms improvement[69]. Although the nonpharmacological treatment has been reinforced in OA management, it is often being undervalued and underutilised in practice. Consensus work and surveys have highlighted the need for health care professionals' training in the skills of supporting self-management and delivering care in line with international guideline recommendations for OA[70 71]. In contrast, pharmacological interventions are widely used to alleviate joint pain when patients seek medical care. The non-steroidal anti-inflammatory drugs (NSAIDs) and opioids use accounts for the majority of pharmacological interventions[72 73]. Efficacy of these medications is effective in pain relief and function restoration[74]. However, these medications have considerable adverse effects, particularly the elderly patients and patients with multiple comorbidities [72 75 76]. Evidence has shown the NSAIDs use associated with fold increase in risks of myocardial infarction (relative risk: 1.4), acute renal failure (relative risk: 3.2), or stroke (relative risk: 1.58) compared with placebo[77-79]. The use of opioids has been shown to raise the risk of all-cause mortality compared with the use of NSAIDs[80].

It should be noted that these treatments are for the reduction of symptoms, and do not provide a disease modification for OA. Most patients in the late-stage of OA with progressive pain and disability are seeking surgery. As a consequence of increasing prevalence, the rate of joint replacement surgery has raised from recent decades. In Australia, the age-standardised rate of total knee and hip joint replacement surgery in hospitalisation increased by 36% and 38% from 2005-2006 to 2015-2016[81]. Apart from the substantial economic burden and no disease modification effect of joint replacement surgery, there are still limitations remaining post-surgery[82]. Continuing to experience pain in the replaced joint, post-surgery mortality, infection, stiffness, loss function of scar tissue and other complications are needed to consider.

There are currently no existing treatments that can slow or halt the disease progression of OA. Moreover, these available treatments are related to adverse events or high economic burden. Therefore, there is an urgent need for clinical studies to develop cost-effective treatments that may intervene in the pathophysiology and progression of OA.

1.1.4 Comorbidities

For older adults, OA is the disease with the highest co-prevalence of other chronic diseases or long-term conditions[8]. The WHO defined this condition as "comorbidity". Various epidemiological studies have demonstrated that people with OA are at significantly higher risk of developing comorbidity than without OA[83]. In Australia, 2014–15, 79% people of all ages with arthritis reported having at least one other chronic condition[25]. Over half of elderly patients with arthritis had hypertension, followed by chronic cardiovascular disease, dyslipidaemia, diabetes and mental health problems/depression[84]. Besides, OA co-existing with other musculoskeletal conditions are prevalent[83]. A better understanding of comorbidity and identifying modifiable risk factors in OA are crucial and critical areas for intervention.

Depression is a major global public health issue and is projected to be the second leading cause of disease burden by the year 2020[85]. In addition, depression is prevalent comorbidity in OA patients. A recent systematic review and meta-analysis reported that 19.9% of people with OA had depressive symptoms, which is almost twofold of the prevalence in the US general population, with a relative risk of depression of 1.17 in those with OA compared to those without[86 87]. Furthermore, suffering from concomitant depression in OA patients contributes to its increased difficulties in OA management and disease burden[9].

Foot pain is typical and very common musculoskeletal pain, especially in older adults[88-90]. It affects nearly one in five older people in the community and people who reported knee pain were more likely to report foot pain[91]. A survey included 8990 older people reported that most people with knee pain had multiple joint site pain, and the severity of knee pain and related disability were worse in the presence of pain elsewhere[92]. More important, foot pain is also prevalent in knee OA patients[93]. In the OA initiative cohort study, 25% knee OA patient reported occurrent foot pain. Foot pain has a significant detrimental impact on health-related quality of life and physical activity[10 94], while foot pain coexists with knee pain leading to even worse physical activity and lower quality of life in knee OA patients[95 96].

In summary, comorbidities are very common in OA patients and coexisted comorbidity exerts significant impacts on individuals and health care systems[11 97]. Therefore, screening, prevention and treatment of comorbidity in OA patients should not be taken lightly.

1.2 Vitamin D and osteoarthritis

Vitamin D is a group of fat-soluble secosteroid. Most foods except oily fish, cod liver oil and mushrooms contain little vitamin D unless fortified and few vitamin D_3 can be taken from diet[98]. More than 90% of the vitamin D being synthesized in the skin for most people comes from casual exposure to solar ultraviolet B radiation[99]. Hence, vitamin D has been recognised as sunshine vitamin. Vitamin D originating from the diet or the skin enters the circulation and is metabolized to 25-hydroxyvitamin D [25(OH)D] by the 25-hydroxylase in the liver and then to the hormonal form 1,25-hydroxyvitamin D [$1,25(OH)_2D$] by the 25-hydroxyvitamin D-1α-hydroxylase in the kidney[100]. 25(OH)D is the major form in the circulation, which determines people's vitamin D status and the $1,25(OH)_2D$ is the active form, which is the ligand for the vitamin D receptor (VDR), altering the transcription rates of target genes responsible for the biological responses[101].

1.2.1 Importance of vitamin D

Emerging evidence has shown the vitamin D may have a promising effect on OA and its comorbidities for the reason that vitamin D can exert benefits beyond the skeletal benefit. Brain, prostate, breast, and colon tissues, among others, as well as immune cells, have a VDR and respond to $1,25(OH)_2D$[102]. Similar to other steroid hormones, $1,25(OH)_2D$ binding to the VDR is the initial step to activate the VDR to induce or repress the expression of genes. In addition, some of these tissues and cells also express the enzyme 25-hydroxyvitamin D-1α-hydroxylase that have the ability to produce $1,25(OH)_2D$[103].

The ability of $1,25(OH)_2D$ to inhibit growth and promote differentiation of a variety of cell types has suggested diverse functions in preventing cancers, modulating the immune system, and controlling various endocrine systems[101 103]. This is in line with epidemiological evidence that vitamin D can play an important role in decreasing the risk of cancers, autoimmune diseases, infectious diseases, and cardiovascular disease[104]. Vitamin D deficiency is linked to increased incidence of some diseases, including colorectal cancer, type 1 diabetes, multiple sclerosis and hypertension[105-108]. Furthermore, vitamin D deficiency is also associated with OA and other musculoskeletal pain, as well as depression.

1.2.2 Prevalence of vitamin D deficiency

Vitamin D deficiency is the most common nutritional deficiency worldwide[109]. The pathogenesis of vitamin D deficiency appears to be the result of a complex interplay between mechanical, cellular, and biochemical forces. Circulation level of 25(OH)D is recommended to evaluate vitamin D status[110]. Although vitamin D deficiency is common, there is no consensus to define vitamin D deficiency measured by 25(OH)D[111]. The Institute of Medicine has reviewed available literature and recommended serum 25(OH)D level of less than 50 nmol/litre (20ng/ml) as vitamin D deficiency[112].

With the definition, it has been established that 1 billion people have vitamin D deficiency or insufficiency[113 114]. Several national cohorts have suggested the prevalence of vitamin D deficiency. The overall prevalence rate of vitamin D deficiency in the US was 41.6%[115]. In Canada, 57.5% of men and 60.7% of women had a level of less than 75 nmol/liter[116]. Nearly one-third of Australian adults aged than 25 years old had vitamin D deficiency and 73% Australian had vitamin D insufficiency based on epidemiology evidence[117]. Furthermore, vitamin D deficiency is widespread throughout the European population, 13.0% European individuals had serum 25(OH)D below than 30 nmol/litre and 40.4% had vitamin D deficiency[118]. Similar to OA, female, elderly people and obesity/overweight population are more likely to develop vitamin D deficiency[119 120].

1.2.3 Vitamin D and OA

There is increasing evidence for a potential role of vitamin D in OA pathogenesis[121]. Vitamin D may have direct effects on chondrocytes. The chondrocytes in the articular cartilage are important for synthesis and repair of the extracellular matrix in cartilage[122]. Loss of homeostatic balances between the anabolic and catabolic processes in chondrocytes leads to the failure of articular cartilage and lead to the progression of OA[123]. Matrix metalloproteinases (MMPs) are known to be important in regulating cartilage homeostasis[124]. VDR has been demonstrated expressed in human osteoarthritic chondrocytes and more than health cartilage, especially the superficial zone[125]. The presence of VDR expression could regulate MMPs expression, especially MMP-1, MMP-3, MMP-9 and MMP-13, which are responsible for and

resulting in bone and cartilage degradation[125-127]. This suggested that articular cartilage is sensitive to the effect of vitamin D.

Vitamin D may also exert an effect on OA through regulation of bone homeostasis between bone formation by osteoblasts and bone resorption by osteoclasts[128 129]. Alterations in the pathological processes in bone, including subchondral bone remodelling, increased bone turnover and bone expansion, which lead to the initiation and progression of OA[130-132]. Additionally, vitamin D may affect OA through inhibition of inflammation. Inflammation has been implicated in the pathogenesis of OA as increased inflammatory and/or metabolic biomarkers[133 134]. Vitamin D may reduce the inflammatory response through modulating human monocyte function or VDR signalling[135-137]. Hence, increased inflammation may be a key underlying mechanism linking vitamin D deficiency to OA, and vitamin D could modify OA disease progression through inhibition of inflammation. In summary, vitamin D may have the potential to delay the development and progression of OA.

1.2.3.1 Evidence from prospective studies

Although the findings from high-quality prospective studies about the association between vitamin D deficiency and OA were inconsistent (Table 1.2), strong evidence had suggested vitamin D deficiency is linked with the risks of cartilage loss, when joint space loss and change in cartilage volume measurement were combined, in knee OA[30 138-141]. In addition, there was evidence that low levels of 25(OH)D were associated with increased progression of ROA, but not incident ROA assessed by the K&L score system and change in focal cartilage defects. However, there are inconsistent results in the association between vitamin D deficiency and joint pain resulting from OA and only one cohort study supported the association between 25(OH)D levels and the prevalence or incidence of symptomatic OA[142 143]. Due to vitamin D deficiency related to the risk of cartilage loss and progression of ROA, greater attention to the important role of vitamin D deficiency in OA pathogenesis is required.

Table 1.2 Cross-sectional and longitudinal studies for the association between 25(OH)D and OA

Study Cohort	Participants number	Age range	Female	Follow-up years	Assessment of OA	Results	Conclusions
Framingham OA Study [138]	556	70.3±4.5	57%	8	Modified K&L score (0-4), JSN (0-3) and osteophytes (0-3)	Low serum level 25(OH)D predicted loss of cartilage, as assessed by loss of joint space (OR: 2.3, 95% CI: 0.9 to 5.5) and osteophyte growth (OR: 3.1, 95% CI: 1.3 to 7.5).	Low serum levels of 25(OH)D were associated with increased risk for progression of knee OA.
Osteoporotic Fractures Study[139]	237	71	100%	8	Hip JSN (0-4), osteophytes (0-3) and a summary grade (0-24) of hip ROA	Participants in the middle (OR: 3.21, 95% CI: 1.06, 9.68) and lowest (OR: 3.34, 95% CI, 1.13, 9.86) tertiles for 25(OH)D associated with increased incidence of hip JSN compared with participants in the highest tertile.	Low serum levels of 25(OH)D were associated with the increased incident changes of hip JSN.
Framingham OA Study and the Boston OA of the Knee Study[144]	992	53.1±8.7 and 66.2±9.3	53% and 41%	9.5 and 2.5	Modified K&L score (0-4), JSN (0-3) and osteophytes (0-3);	No association of baseline 25(OH)D levels with radiographic worsening	Vitamin D status was not associated with the

					MRI, WORMS assessment for cartilage loss	in either cohort. 25(OH)D was unrelated to cartilage loss.	risk of joint space and cartilage loss.
Tasmanian Older Adult Cohort (TASOAC) study[140]	880	51 to 79	50%	2.9	MRI measurement for cartilage volume and defects	Baseline serum 25(OH)D level was positively associated with change in cartilage volume and change in serum 25(OH)D level was positively associated with change cartilage volume.	Achieving vitamin D sufficiency may prevent and/or retard cartilage loss in knee OA.
Rotterdam Study[141]	1248	66.2±6.7	58%	6.5	K&L score (0-4), JSN and osteophytes	Progressive ROA occurred more in participants with the lowest tertile against the highest tertile of vitamin D intake (OR: 7.7, 95% CI, 1.3, 43.5). 25(OH)D was significantly related to incident JSN in subjects with low lumbar spine BMD at baseline.	Vitamin D status seems to influence the incidence and progression of knee ROA.
Mini-Finland Health Survey[142]	805	30 to 50	55%	22	Definite and probable knee and hip OA	No significant association between serum 25(OH)D level and the risk	Low level of serum 25(OH)D may not

					diagnosed by a clinical examination or by physicians	of incident knee or hip OA. However, the risk of low serum 25(OH)D level increased significantly in winter (OR: 1.57; 95% CI: 1.10, 2.27).	contribute to the development of OA.
Osteoarthritis Initiative Study[145]	418	61.0±9.2	47%	2	OARSI Atlas, JSN (0-3), progression in JSN score	Participants with low vitamin D had increased risk of knee OA progression compared with those with greater 25(OH)D concentration (OR: 2.3; 95% CI: 1.1, 4.5).	Individuals with vitamin D deficiency have an increased risk of knee OA progression.

OR, odds ratio; 95% CI, 95% confidence interval;

WORMS, Whole-Organ Magnetic Resonance Imaging Score;

BMD, bone mineral density.

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1.2.3.2 Evidence from clinical trials

There are several clinical trials that have examined the effect of vitamin D supplementation on OA but did not provide consistent results to suggest whether OA patients should have vitamin D supplementation[143].

McAlindon et al reported no effect of vitamin D supplementation (vitamin D3, 2000 IU/day over two years) on cartilage volume loss or knee pain in patients with knee OA [146]. However, the major limitations of this study were small sample size (n=146), the inclusion of participants with both vitamin D sufficiency and insufficiency, and the inclusion of participants with severe disease[147]. Participants with sufficient vitamin D may not benefit from vitamin D supplementation, and patients with severe disease would not respond to any treatment[147]. Consistently, a recent trial reported that vitamin D supplementation for three years did not slow progression of JSN and reduce Western Ontario and McMaster Universities OA Index (WOMAC) pain, stiffness and function in knee OA[148]. In contrast, another RCT reported that vitamin D supplement at a high dose (60,000 IU per day for ten days followed by 60,000 IU once a month for a year) reduced knee pain and improved function in knee OA, but this study was limited by its small sample size and short follow-up period [149].

The VIDEO study in Australia overcame the limitations of these studies and aimed to determine whether monthly supplementation with 50,000 IU vitamin D over 24 had beneficial effect on WOMAC pain and cartilage volume loss, but found largely consistent results that vitamin D supplementation did not have significant effects on cartilage volume loss or WOMAC pain in patients with knee OA[150]. However, in the secondary analysis, the data have shown that the intervention group had statistically significant improvements in visual analogue scale (VAS) knee pain and WOMAC function when compared with the placebo group. The VIDEO study also reported that compared with placebo, vitamin D supplementation significantly reduced MRI-measured joint effusion-synovitis in patients with symptomatic knee OA[151]. This suggests that vitamin D supplementation could have anti-inflammatory effects in knee OA patients. While there were 62% participants reached a higher level of 25(OH)D (>50 nmol/l) at month 24 in the placebo group, it would dilute the beneficial effects of vitamin D supplementation.

Inconsistent results of these clinical trials would be due to variations in factors such as inclusion criteria of participants, disease severity, vitamin D doses, duration of the treatment, and

outcome measures. Therefore, whether vitamin D has beneficial effects on OA and whether inflammation is potential mechanisms linked to vitamin D and OA need to be explored in future studies.

1.2.4 Vitamin D and OA comorbidities

Vitamin D deficiency is associated with a range of mental disorders including depression[152]. The underlying biological mechanism is not clearly understood. It has been demonstrated the widespread distribution of VDR and 25-hydroxyvitamin D-1α-hydroxylase in the human brain and suggested the potential effect of vitamin D on maintaining normal brain function[153 154]. Researches have reported low vitamin D can negatively affect growth, cellular signalling and neural activity, which may be an underlying mechanism that contributes to depression[155]. Vitamin D insufficiency is associated with increased inflammation, which also may play an important role in the mechanism for depression[156 157]. Furthermore, insufficient vitamin D level is not optimal for active serotonin synthesis and releasing, leading to the development of depression[158].

Evidence from observational studies have demonstrated low vitamin D concentration is associated with depression[159]. Although the biological mechanisms underlying the associations between vitamin D deficiency and depression are unclear, the therapeutic potential of vitamin D supplementation for depression has been investigated in the general population and patients with other chronic diseases, except for OA[160]. Up to now, evidence from randomised controlled trials (RCT) was contradictory. Considerable heterogeneity of study characteristics and intervention effects among studies were observed[160]. Conducting trial in participants without depression or/and vitamin D deficiency may explain why most studies did not find clear benefits of vitamin D supplementation[161-164]. Further well-designed vitamin D supplementation RCTs among individuals who are both depressed and vitamin D deficient are needed.

The emergence of experimental data suggests vitamin D may exert anatomic, hormonal, neurological and immunological influences on pain manifestation, thereby playing a key role in the aetiology and maintenance of chronic pain with complicated mechanisms[165-167]. Vitamin D can modulate neuronal excitability, influences prostaglandin, inhibits synthesis of nitric oxide synthase, upregulates synthesis of neurotrophins, and affects a number of inflammatory

pathways, which are associated with chronic pain[168-171]. These potential mechanisms may be involved the vitamin D deficiency and chronic pain. However, clinical research regarding the relationship between vitamin D deficiency and chronic pain remained limited and did not provide consensus.

Previous studies have found that low levels of vitamin D are associated with chronic pain[167 172 173], but there was only one cross-sectional cohort study to examine the association between vitamin D status and foot pain. The population-based study has reported lower level vitamin D was not related to foot pain, but back pain[174]. However, on account of the nature of the cross-sectional study, the causal relationship was not sure. Also, no study has explored the effect of vitamin D on foot pain. Even so, vitamin D deficiency and foot pain are prevalent in OA patients; it is also worthwhile to examine the effect of vitamin D on foot pain in future.

1.3 Summary

There is clear evidence that OA is a progressive and serious disease, which is associated with increased risk of progressive disability and increased risk of co-occurrence of comorbidity. As increasing OA risk factors, including the aging population, increasing obesity rate, the prevalence of OA is expected to become higher. Thus, OA has substantial impacts on individuals and the health system. However, currently, there is no approved treatment which could delay the disease progression of OA.

Experimental and epidemiological studies have shown vitamin D deficiency is a modifiable risk factor for disease onset and disease progression. Given that there are inconsistent results from clinical trials, whether vitamin D has beneficial effects on OA and the potential mechanisms linked vitamin D to OA needs to be explored in future studies.

Depression and foot pain are prevalent comorbidities in OA patients. Co-occurrence with a commodity in OA patients is projected to increase difficulties for management and disease burden. Researchers and clinicians should pay more attention to the screening, prevention and treatment of comorbidity in OA patients. To identify modifiable risk factors systemically for OA comorbidity are essential for disease prevention and treatment. In term of potential effects

of vitamin D on OA comorbidity, few studies have been conducted in OA patients. Therefore, clinical studies are needed.

Chapter 2 Research Questions

The background and rationale of this thesis have been reviewed in Chapter 1.

The research questions of this thesis are summarized as follows:

1. To examine whether maintaining sufficient serum vitamin D levels is associated with changes in joint structures and symptoms in patients with symptomatic knee OA and baseline vitamin D insufficiency over two years.
    a. Whether maintaining sufficient serum vitamin D levels has an association with joint structural change over two years?
    b. Whether maintaining sufficient serum vitamin D levels has an association with symptomatic joint change over two years?
2. To determine whether vitamin D supplementation or variation in vitamin D status affected serum inflammatory and metabolic biomarkers in patients with symptomatic knee OA and vitamin D insufficiency over two years.
    a. Does vitamin D supplementation affect serum inflammatory and metabolic biomarkers over two years?
    b. Is variation in vitamin D status associated with serum inflammatory and metabolic biomarkers change over two years.
3. To evaluate the effect of vitamin D supplementation and maintaining sufficient serum vitamin D levels on depressive symptoms in patients with symptomatic knee OA and vitamin D insufficiency over two years.
    a. Does vitamin D supplementation have a beneficial effect on depressive symptoms?

   b. Whether maintaining sufficient serum vitamin D levels has an association with depressive symptoms?
4. To describe and explore the prevalence, incidence and potential risk factors of depression in patients with symptomatic knee OA.
   a. To describe demographic and clinical factors associated with the prevalence and incidence of depression in patients with symptomatic knee OA.
   b. To explore the temporal relationship between depression and joint symptoms in patients with symptomatic knee OA.
5. To evaluate the effect of vitamin D supplementation and maintaining sufficient serum vitamin D levels on foot pain in patients with symptomatic knee OA and vitamin D insufficiency over two years.
   a. Does vitamin D supplementation have a beneficial effect on foot pain revelling?
   b. Whether maintaining sufficient serum vitamin D levels has an association with foot pain.

Chapter 3 Methodology

All chapters are derived from analyses using the data from the Vitamin D Effect on Osteoarthritis (VIDEO) study, which is a randomised clinical trial and aims to examine the effects of vitamin D supplementation on knee pain and knee structural changes in symptomatic knee OA patients with low serum vitamin D levels. This chapter is to describe the VIDEO study. It comprises aspects of study design, participants, intervention and outcome measures, and the methods of statistical analyses.

Please note that the subsequent data chapters are presented in the form in which they were accepted by or submitted to peer-reviewed scientific journals. Therefore, there are some differences in the way that are presented in those papers as compared to this overview chapter due to the requirements of different journals and the details required for different analyses.

The sample sizes used in individual chapters vary for each of the research questions. Additional factors which are unique to each chapter are described in more detailed in the methodology section of each of the subsequent chapters.

3.1 Study design, setting and ethics

The VIDEO study is a randomised, placebo-controlled double-blind clinical trial, which was conducted in the Hobart and Melbourne.

Recruitment methods included advertisements through the local media and community groups as well as liaisons with general practitioners, specialist rheumatologists, and orthopedic surgeons. A telephone pre-screening was conducted to inquire about knee pain status, comorbidities, participation in other studies, and whether the survey recipient anticipated knee or hip surgery within the next two years. Potentially eligible participants were subsequently assessed during a clinic visit that included a physical examination, knee radiography, and assessment of serum 25-hydroxyvitamin D levels.

3.1.1 Participants

Participants with symptomatic knee OA and serum 25(OH)D> 12.5 nmol/litre and < 60 nmol/litre was recruited. The participant inclusion and exclusion criteria for the VIDEO study are listed following[175]:

Inclusion criteria:

1. Age 50-79 years old;
2. Men and women with symptomatic knee OA for at least 6 months with a pain VAS of at least 20 mm;
3. Meet the ACR criteria for symptomatic knee OA assessed by a rheumatologist[18];
4. Have an ACR functional class rating of I, II and III[176];
5. Have relatively good health (0-2 according to the investigators global assessment of disease status on a 5-point Likert scale, range 0 [very well] to 4 [very poor]); and
6. Have serum vitamin D level of >12.5 nmol/L and <60 nmol/L.
7. Is able to read, speak and understand English, capable of understanding the study requirements and willing to co-operate with the study instructions.

Exclusion criteria:

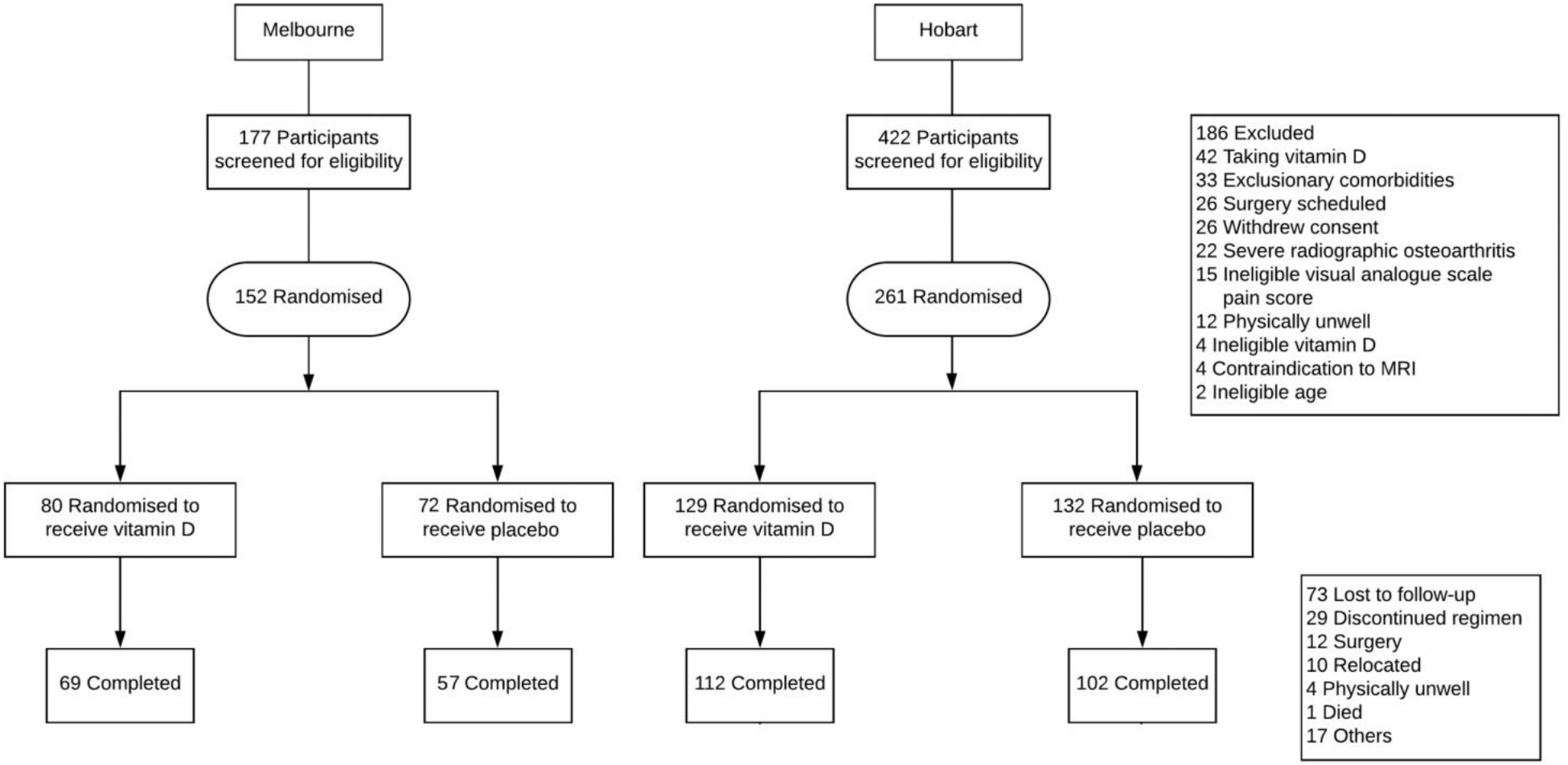
1. Patients with severe radiographic knee OA (grade 3 according to Altman's atlas)[177 178];
2. Patients with severe knee pain (on standing more than 80 mm on a 100-mm VAS);

3. Any contra-indication to having an MRI.
4. Patients with rheumatoid arthritis, psoriatic arthritis, lupus, or cancer;
5. Patients with severe cardiac or renal function impairment
6. Patients with hypersensitivity to vitamin D;
7. Patients with any condition possibly affecting oral drug absorption (e.g. gastrectomy or clinically significant diabetic gastro-enteropathy);
8. having significant trauma to the knees including arthroscopy or significant injury to ligaments or menisci of the knee within 1 year preceding the study;
9. having anticipated need for knee or hip surgery in the next 2 years;
10. having taken Vitamin D supplements in last 30 days;

A total of 599 participants were screened for eligibility, and 413 participants were recruited from June 2010 to December 2011. Informed written consent was obtained from all participants. Participants were randomly allocated to either the intervention or placebo group.

The flowchart provides an overview of participant recruitment and withdrawal during the study period.

Figure 3.1 Flowchart of the VIDEO study



3.1.2 Randomisation

Participants were allocated to either the vitamin D or placebo arm at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a centrally automated allocation procedure with security in place to ensure allocation data could not be accessed or influenced by any person from the investigative team.

Participants, research coordinators and investigators were all blinded to treatment assignment. Blinding was maintained until all data were collected, cleaned, confirmed for accuracy and analyses of primary outcomes were performed.

3.1.3 Sample size

The sample size calculation was based on the Cohen formula and assumed would give 80% power with a 5% probability of type 1 error (α= 0.05 and β= 0.20)[179].

Data from previous studies reported that mean annual medial tibial cartilage volume loss in knee OA patients was 4.5%[48]. Monthly supplementation with 50,000 IU vitamin D would correct vitamin D deficiency, and this change was estimated to lead to an absolute reduction in medial tibial cartilage loss of 2.2% annually, which was expected to reduce 44% risk for total knee replacement over four years[41 48 140 180]. Based on these data, with 400 participants at baseline (200 in each group), allowing 20% dropouts, would have at least 80% power to detect a 2.2% between-group difference in medial tibial cartilage loss.

Data from previous vitamin D clinical trial, it provides a standard deviation (SD) estimate of 70.5 on the WOMAC pain scale (0 to 500)[149]. The minimal clinically important difference (MCID) for WOMAC pain was previously reported to be 16% reduction of the score from baseline[181]. The sample with 400 participants to detect a difference between groups of 20 units on the score is powerful.

3.1.4 Ethics

The Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182 - 2010000616) approved the ethics of this study.

3.1.5 Trial registration

ClinicalTrials.gov identifier: NCT01176344

Anzctr.org.au identifier: ACTRN12610000495022

3.2 Intervention

Participants were received 50,000IU (1.25mg) oral vitamin D3 capsule (cholecalciferol) monthly for 24 months or an identical inert placebo[175]. Both the vitamin D3 compound and placebo were prepared by and purchased from Nationwide Compounding Pharmacy, Melbourne, Australia. All inform participants were contacted to remind them to take their capsule monthly. Adherence to vitamin D capsules was calculated by recording how many capsules were left when the participants were asked to take the capsules with them at follow-up visits.

3.3 Outcomes measurement

Primary outcome measures of the VIDEO study were changed in knee pain, assessed using the WOMAC score and changed in tibial cartilage volume, assessed on MRI from baseline to month 24. Secondary outcomes included VAS knee pain, lower limb muscle strength and other structural changes on MRI. A full list of outcome measures for the VIDEO study is shown in Table 3.1.

Table 3.1 Outcomes measurement and timetable of the VIDEO study

	Screening	Months				
		0	**3**	**6**	**12**	**24**
MRI		✓				✓

Knee pain (VAS and WOMAC)	✓	✓	✓	✓	✓	✓
Knee radiograph	✓					
Serum 25-(OH)D	✓		✓			✓
Serum calcium, phosphate, creatinine	✓		✓			
Micro-CT (in Melbourne)		✓				✓
DXA (in Hobart)		✓				✓
Core musculature measure		✓			✓	✓
Lower limb muscle strength		✓	✓	✓	✓	✓
Hand grip strength		✓	✓	✓	✓	✓
Weight		✓	✓	✓	✓	✓
Height		✓				✓
Skin fold		✓				✓
Girth measurements		✓				✓
Upper arm pressure		✓	✓	✓	✓	✓
Central blood pressure		✓				✓
Ambulatory blood pressure		✓				✓
Arterial stiffness		✓				✓
Physical activity (IPAQ)		✓				✓
Medications	✓	✓	✓	✓	✓	✓
Sun exposure		✓		✓	✓	✓
Low back pain		✓	✓	✓	✓	✓
Foot pain		✓	✓	✓	✓	✓
Depression		✓	✓	✓	✓	✓
Quality of life		✓	✓	✓	✓	✓
Cigarette smoking		✓				✓
Previous knee injury		✓				✓
Occupation		✓				✓
Pill counts		✓	✓	✓	✓	✓

Adverse events	✓	✓	✓	✓	✓

If the participant withdraws after a minimum of 6 months of treatment, he/she was requested to have a second knee MRI (HR-pQCT or DXA) scan.

3.3.1 Anthropometrics

Body weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707, Bradford, MA, USA). Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. BMI was calculated as kg/m^2.

3.3.2 Demographic characteristics

Age and gender were recorded at baseline. Participants filled out a questionnaire which collected information on education history (grade 0: less than high school, grade 1: high school degree and grade 2: superior than high school degree), current regular smoker (yes or no) and concomitant medication usage.

3.3.3 Serum 25(OH)D levels measurement

Serum 25(OH)D was measured at baseline, month 3 and 24 using direct competitive chemiluminescent immunoassays, which is an accurate and reproducible automated tool (DiaSorin Inc.)[182]. The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%, respectively[150]. The season of blood sample was recorded.

3.3.4 Joint symptoms assessment

Knee symptoms were assessed at baseline and months 3, 6, 12, and 24 using WOMAC and the VAS scale[183 184]. The total WOMAC score (0-2400) is the sum of subscale scores including pain (0-500), stiffness (0-200), and physical function (0-1700), which is a widely used instrument to evaluate the functional capacity in patients with OA and demonstrates high performance in the clinical trial. Knee pain will be assessed by both WOMAC pain with five subscales in 100 mm visual analog format (walking on a flat surface, going up/down stairs, at night in the bed, sitting/lying and standing upright).

And knee pain in most days of the previous month was also assessed using a 100 mm VAS. The VAS consists of a straight line with the endpoints defining extreme limits such as "no pain at all" and "pain as bad as it could be"[185]. Participants were asked to mark his pain level on the line between the two endpoints. VAS has been demonstrated to be sensitive to treatment effects[186].

3.3.5 Joint structures assessment

3.3.5.1 MRI detected joint structures

MRI scans of the study knee were obtained according to a standardised protocol at baseline and month 24, using a 1.5 T whole-body MRI unit with a commercial transmit-receive extremity coil at baseline and two years. The sequences were used as follow:

(1) T1-weighted fat saturation 3D gradient recall acquisition in the steady state, flip angle 30°, repetition time 31 ms, echo time 6.71 ms, field of view 16 mm, 60 partitions; 512×512 matrix; acquisition time 5 min 58 s. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31×0.31 (512×512 pixels).

(2) T2-weighted/proton fat saturation 3-D fast spin echo sequence, flip angle 90, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228×256-pixel matrix; sagittal images were obtained at a partition thickness of 2 mm with a between-slices gap of 0.5 to 1.0 mm.

The image database was transferred to a computer workstation, the Osirix imaging software[187] [188]. MRIs were assessed by trained readers blinded to treatment allocations according to methods described previously[175]. Baseline and follow-up MRIs were scored in pairs in chronological order to minimise measurement error[189].

Knee tibial and patellar cartilage volumes were measured on T1-weighted MRI by a single trained observer using the previously described image processing techniques[190]. The volumes of individual cartilage plates (medial tibial, lateral tibial and patella) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis. Then these data were resampled using bilinear and cubic interpolation (area of 312×312 um

and 1.5 mm thickness, continuous sections) for final 3D rendering using OsiriX imaging software (32-bit version 5.9, Pixmeo SARL). The coefficient of variation (CV) for cartilage volume assessment was ranged from 2.1% to 2.6%[191 192].

Knee cartilage defects were observed on T1-weighted MRI and assessed by a single trained observer using a modification of the Outerbridge classification system (0-4) at patella, tibial and femoral, with details described as following[193-195]: grade 0, normal cartilage; grade 1, focal blistering and low-signal intensity change with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full thickness cartilage loss with exposure of subchondral bone. Cartilage defects score of ≥ 2 at any sites within the compartment was defined as the presence of cartilage defects. Intra-observer reliability was expressed as the intra-class correlation coefficient (ICC), which was ranged from 0.89 to 0.94 and inter-observer reliability was ranged from 0.85 to 0.93[51 190].

Subchondral BMLs, was defined as discrete areas of increased signal adjacent to the subcortical bone one T2 weighted MRI. It was measured using a modification of the classification system of Whole-Organ Magnetic Resonance Imaging Score at tibial (lateral and medial), femoral (lateral and medial) and patella (lateral and medial). The tibial and femoral were divided into three sub-regions (anterior, central and posterior), and the tibial has one additional sub-region which represents the area beneath the tibial spines[196].

BMLs were graded from 0 to 3 based on the extent of sub-regional involvement: grade 0, none; grade 1, ≤ 25% of the subregion; grade 2, 25% to 50%; and grade 3, ≥ 50%. A total score of the tibiofemoral compartment was calculated as the total of 15 subregional scores (0-45). The ICC of this BMLs measurement ranged from 0.93 to 0.98, which was excellent[189 197 198].

Effusion-synovitis were assessed using T2-weighted/proton density–weighted fast spin echo (FSE) sequences and measured at two regions (suprapatellar pouch and other cavity), according to the anatomy of the knee joint synovial cavity[199]. The suprapatellar pouch, extending superiorly from the upper surface of the patellar, between the posterior suprapatellar fat pad (quadriceps femoris tendon) and the anterior surface of the femur. Other cavity includes the

area between the central femoral and tibial condyles, around the ligaments and menisci, and the area behind the posterior portion of each femoral condyle, inside of the joint capsule.

Quantitative assessment of effusion-synovitis volume was measured utilising OsiriX software, which has been described as previously[56]. The volume of joint subregions was isolated from the total volume selecting each region of interest (ROI) according to the intra-articular fluid-equivalent signal on a section-by-section basis and then resampled utilizing bilinear and cubic interpolation for final 3D rendering using OsiriX software. The total area of all ROIs in the same slice was summed to obtain in the suffusion-synovitis area of the slice. The ICCs was ranged from 0.96 to 0.97, and inter-observer reliability was ranged from 0.93 to 0.99.

Semi-quantitative assessment of effusion-synovitis in each subregion was scored individually according to WORMS and was graded collectively from 0 to 3 in terms of the estimated maximal distention of the synovial cavity: grade 0, normal; grade 1, < 33% of maximum potential distention; grade 2, 33% to 66% of maximum potential distention; grade 3, > 66% of maximum potential distention[196]. The presence of effusion-synovitis of the whole joint was defined as a grade of ≥ 2 in any subregion[151]. The inter-rater reliability was ranged from 0.63 to 0.75, and ICC was ranged from 0.60 to 0.75 in different subregions as described previously[57].

3.3.5.2 Radiography

A standing anteroposterior semiflexed view of the studying knee with 15° of fixed knee flexion was performed in all participants. Radiographs were assessed using the atlas developed by Altman et al[177 178]. Each of the followings was assessed on a scale of 0–3 (0= normal, 1= mild or 1-33% abnormal, 2= moderate or 33-66% abnormal and 3= severe or 67-100% abnormal)[200]: medial JSN, lateral JSN, medial femoral osteophytes, medial tibial osteophytes (Figure 3.2), lateral femoral osteophytes, and lateral tibial osteophytes. Each score was determined by consensus of two readers who simultaneously assessed the radiograph with immediate reference to the atlas. ICC ranged from 0.65– 0.85. The presence of ROA was defined as any score ≥ 1 for JSN or osteophytes.

Figure 3.2 Altman altas of knee joint osteophyte

A: grade 0 normal; **B:** grade 1 medial tibal osteophyte; **C:** grade 2 medial tibal osteophyte; **D:** grade 3 medial tibal osteophyte

Source: Altman RD, Gold GE. Atlas of individual radiographic features in osteoarthritis, revised. Osteoarthritis Cartilage 2007;15 Suppl A:A1-56.

Figure 3.3 Altman altas of knee joint space narrowing

A: grade 0 normal; **B:** grade 1 medial tibiodemoral narrowing; **C:** grade 2 medial tibiodemoral narrowing; **D:** grade 3 medial tibiodemoral narrowing

Source: Altman RD, Gold GE. Atlas of individual radiographic features in osteoarthritis, revised. Osteoarthritis Cartilage 2007;15 Suppl A:A1-56.

3.3.6 Physical activity

Physical activity primarily was assessed using a pedometer (SW 200 Digi-Walker, Yamax Corporation, Tokyo, Japan), which measures vertical displacement (steps per day) at baseline and month 24[201]. Briefly, participants were instructed to wear a pedometer for seven consecutive days and to record the number of steps each day and the duration and type of physical activity for any activities in which the pedometer could not be worn (for example, swimming). Mean steps/day was calculated as the average of the days worn at both time points[202].

Physical activity was also measured using the International Physical Activity Questionnaire (IPAQ) short version[203]. It has been developed and tested for use in adults (age range of 18-65 years)[203]. The PA status (insufficiently active, sufficiently active and highly active) was calculated according to the scoring protocol available at http://www.ipaq.ik.se.

3.3.7 Serum inflammatory and metabolic biomarkers

All fasting blood samples were collected at baseline and month 24. The measurements were performed according to the manufacturer's instruction. Serum inflammatory biomarker levels of high-sensitive C-reactive protein (hs-CRP), IL-6, IL-8 and IL-10 were measured. Serum metabolic cytokine levels of resistin, leptin, adiponectin, adipsin and apelin-36 were measured.

Serum hs-CRP was measured by enzyme immunoassays (IBL Inc.). Serum IL-6, IL-8, and IL-10 were measured by Bio-plex Luminex assay kits (Bio-Rad Laboratories Inc.). Serum leptin and apelin-36 were measured by enzyme-linked immunosorbent assay (ELISA, Phoenix Pharmaceuticals Inc.). Serum adiponectin, adipsin, resistin were measured by ELISA (Millipore Inc.). The inter-assay and intra-assay CVs were <10% and < 15% for all inflammatory biomarkers.

3.3.8 Depressive symptoms and depression assessment

Depressive symptoms were assessed using the patient health questionnaire (PHQ-9) at baseline, month 3, 6, 12 and 24. PHQ-9 is a valid and reliable self-reported depression instrument, which

is widely used in multipurpose diagnoses, severity measures in the clinic, as well as assessing depression outcomes in research[204 205].

It is a nine-item questionnaire with a score range of 0 to 27, with each item being scored from 0 to 3 (not at all, several days, more than half days and nearly every day). Using the mental health professional interview as the criterion standard, PHQ-9 scores of 5-9, 10-14, 15- 20 and >20 represent mild, moderate, moderately severe and severe depression, respectively. A PHQ-9 scores ≥ 10 has a sensitivity of 88% and specificity of 88% for major depression[204].

3.3.9 Foot pain assessment

Foot pain was measured using the Manchester Foot Pain and Disability Index (MFPDI) questionnaire at baseline, month 3, 6, 12 and 24. The MFPDI system was developed to measure foot pain and disability in the elderly, observational studies and randomized controlled trials[206 207]. Each item was scored from 0 to 3 (none of the time, on some days and on most days/ every day). The total score was calculated by summing the scores of 17 items and higher score indicated greater foot pain disability.

Four subscales were calculated including functional limitation (items 2-8), pain intensity (items 10-11, 14-17), concern about appearance (items 12-13) and activity restriction (items 1 and 9)[208]. Presence of disabling foot pain was required at least one of the ten functional limitation items (items 1-9,11) experienced as on 'most/every day(s)' in the last month, according to the Roddy et al study[207].

3.4 Statistical analyses

T-tests or chi-squared tests were used to compare the difference in means or proportions as appropriate. Standard diagnostic checks of residuals and model fit comparisons were performed routinely. A p value less than 0.05 (two-tailed) was considered statistically significant.

All statistical analyses were performed on Stata 12.0 for Windows (Stata Corporation, TX, USA). The detailed descriptions of statistical analyses were performed are presented in the relevant chapters.

Chapter 4 Maintaining vitamin D sufficiency and OA

This manuscript has been published (Zheng *et al,* American Journal of Medicine 2017; 130: 1211-1218). The typeset version of the manuscript as it appeared in the journal is in Appendix II. The text of this chapter is the same as the published version, except where changes have been requested by the examiners. Thus, there are some repetitions of the methods.

4.1 Introduction

Osteoarthritis and vitamin D deficiency are very common conditions worldwide, often co-existing, especially in the aging population[121 209]. Osteoarthritis is a major cause of chronic pain and impaired physical function in older adults and has contributed substantially to an increased economic burden and imposed huge challenges on health systems worldwide[210 211].

The ideal treatment for osteoarthritis is to reduce symptoms and slow disease progression. These may, in turn, reduce the impact of osteoarthritis on patients' mobility and quality of life, with a consequent reduction in the need for the joint replacement surgery and the health care costs in the long term[212]. In experimental studies, sufficient vitamin D can protect against increased bone turnover and cartilage degradation[213-215]. While there is increasing epidemiological evidence suggests that insufficient serum vitamin D status is associated with the progression of osteoarthritis and worsening in its symptoms, the results have been inconsistent[30].

In the Vitamin D Effect on Osteoarthritis (VIDEO) randomised controlled trial, we reported that vitamin D supplementation did not prevent tibial cartilage loss or improve knee pain as assessed using the Western Ontario McMaster Osteoarthritis Index (WOMAC), but had significant but small effects on visual analog scale (VAS) knee pain, total WOMAC score and WOMAC function in post-hoc analyses in participants with symptomatic knee osteoarthritis and insufficient vitamin D levels[150]. Although the level of 25-hydroxyvitamin D (25(OH)D) increased much more in the vitamin D group (40.6 nmol/L) than in the placebo group (6.7 nmol/L) over two years, 62% participants in the placebo group still reached a sufficient level of serum 25(OH)D (>50nmol/l) at month 24 (unpublished data). Thus, the high proportion of participants achieving sufficient 25(OH)D level in the placebo group may have masked the beneficial effects of vitamin D supplementation.

Therefore, we conducted a post-hoc analysis of the VIDEO study to describe whether maintaining sufficient serum vitamin D levels in people with knee osteoarthritis and baseline vitamin D insufficiency had an association with change in knee structures and symptoms over two years.

4.2 Materials and Methods

4.2.1 Participants

This study is a post-hoc analysis of the VIDEO study. VIDEO was a multicentre, randomized, double-blind, placebo-controlled clinical trial in Tasmania and Victoria, Australia, which aimed to evaluate the effect of vitamin D supplementation over two years on knee pain and knee cartilage volume in people with symptomatic knee osteoarthritis combined with low 25(OH)D levels. Trial design, inclusion and exclusion criteria have been described in the published protocol[175]. Participants had symptomatic knee osteoarthritis (assessed using American College of Rheumatology, ACR criteria[18]) at least for 6 months and had the pain of >20 mm on a 100-mm VAS with low levels of 25(OH) D (between 12.5 and 60 nmol/L). Participants with severe radiographic changes (grade 3 of Altman and Gold Atlas[178]), severe knee pain on standing (>80mm on a 100-mm VAS), contraindications to magnetic resonance imaging (MRI), rheumatoid or psoriatic arthritis, lupus, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, conditions affecting oral drug absorption, anticipated knee or hip surgery within the next 2 years, history of significant trauma of knees (e.g. arthroscopy or injury to ligaments or menisci within one year preceding the study) and history of taking vitamin D or an investigational drug within the last 30 days were excluded[175]. 413 participants aged 63.2 years old were included and 340 completed the study with serum 25(OH)D levels measured at month 3 and 24.

4.2.2 Vitamin D measurement and groups

Serum 25(OH)D was measured at baseline, month 3 and 24 utilizing direct competitive chemiluminescent immunoassays (DiaSorin Inc.). The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%, respectively[150]. Serum 25(OH)D levels of ≤50 nmol/l was defined as vitamin D deficient, and of >50 nmol/l defined as vitamin D sufficient[111 216]. Participants for the current analysis were classified into three groups based on the levels of 25(OH)D at month 3 and 24: consistently insufficient (serum 25(OH)D≤50nmol/l at both month 3 and 24), fluctuating (serum 25(OH)D>50nmol/l at either point) and consistently sufficient (serum 25(OH)D>50nmol/l at both month 3 and 24) vitamin D groups.

4.2.3 Assessment of knee structural changes

MRI scans of the study knee were obtained according to a standardized protocol using a 1.5 T whole-body MRI unit with a commercial transmit-receive extremity coil at baseline and two years. The sequences used for cartilage volume assessment were sagittal fat-saturated T1-weighted spoiled gradient echo. Cartilage defects, bone marrow lesions and effusion-synovitis volume were assessed using T2-weighted/proton density–weighted fast spin echo sequences. MRIs were assessed by trained readers blinded to treatment allocations according to methods described previously[42 175 217].

Cartilage volume was determined using the previously described image processing techniques[175]. The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled using bilinear and cubic interpolation for final three-dimensional rendering using OsiriX imaging software (32-bit version 5.9, Pixmeo SARL). The coefficient of variation was 2.1% for medial tibia and 2.2% for the lateral tibia.

Cartilage defects (0-4) were graded using a modification of the Outerbridge classification system at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, with details described in the protocol[193]. A total score of the tibiofemoral compartment was calculated as the total of 2 subregional scores (medial tibial and femoral, lateral tibial and femoral, 0-8). Intra-observer reliability expressed as an intra-class correlation coefficient ranged from 0.77 to 0.94.

Bone marrow lesions, defined as discrete areas of increased signal adjacent to the subcortical bone, were measured using a modification of the classification system of Whole-Organ Magnetic Resonance Imaging Score (0 =none, 1 ≤25% of the subregion, 2 =25%-50%, and 3≥ 50%)[217]. A total score of the tibiofemoral compartment was calculated as the total of 12 subregional scores (0-36). The intra-class correlation coefficient of this bone marrow lesions measurement ranged from 0.93 to 0.98.

Effusion-synovitis volume at 4 regions (suprapatellar pouch, central portion, posterior femoral recess and subpopliteal recess) were isolated from the total volume selecting each region of

interest according to the intra-articular fluid-equivalent signal on a section-by-section basis and then resampled by means of bilinear and cubic interpolation for final 3D rendering using OsiriX software[218 219]. The intra-class correlation coefficient was from 0.96 to 0.97.

Change in cartilage volume and effusion-synovitis volume were calculated as follows:

$$\text{Absolute change (ml)} = Follow\ up\ volume - Baseline\ volume$$

$$\text{Percentage change per annum (\% p.a.)} = (Absolute\ change)/(Baseline\ volume * Time\ between\ 2\ scans\ in\ year) * 100$$

Change in cartilage defects and bone marrow lesions were calculated as follow:

$$\text{Cartilage defects change} = \text{Follow up defects} - \text{Baseline cartilage defects}$$

$$\text{Bone marrow lesion change} = \text{Follow up bone marrow lesions} - \text{Baseline bone marrow lesions}$$

4.2.4 Assessment of symptomatic changes

Knee symptoms were assessed at baseline and months 3, 6, 12, and 24 using WOMAC and the VAS[183 184]. The total WOMAC score (0-2400) is the sum of subscale scores including pain (0-500), stiffness (0-200), and physical function (0-1700).

4.2.5 Anthropometrics and questionnaires

Height was measured to the nearest 0.1 cm (with shoes removed) using a stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Leicester, UK). Weight was measured to the nearest 0.1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307, Heine, New Hampshire, USA). Body mass index (kg/m^2) was calculated. We also recorded the use of nonsteroidal anti-inflammatory drugs in VIDEO study.

4.2.6 Statistical Analysis

The one-way ANOVA or Kruskal-Wallis rank tests were used to compare differences in baseline characteristics (age, sex, body mass index, cartilage volume, cartilage defects, bone marrow lesions, effusion-synovitis volume, VAS scores and WOMAC scores) among three vitamin D groups. To take into account missing data, we assumed data were missing at random and used a weighted estimating equation method[220 221]. We estimated the probability of a participant remaining in the study during follow-up by fitting a logistic regression model using the baseline characteristics age, sex, body mass index and level of 25(OH)D as predictors, for which complete data were available. In subsequent analyses, completed cases were weighted by the inverse of their estimated probabilities of being observed. Univariable and multivariable linear regressions were used to examine the difference in the changes in cartilage volume, cartilage defects, bone marrow lesions and effusion-synovitis volume between vitamin D groups before and after adjustment for age, sex, body mass index, change in season of blood sampling. The difference in changes of symptoms between different vitamin D groups was analysed using a repeated-measures mixed model with terms for age, sex, body mass index and season of blood sampling. The correlation between the repeated measures was addressed by using individual participant identification as a random effect. The differences between groups were further adjusted for use of nonsteroidal anti-inflammatory drugs. We used Stata 12.0 for Windows (Stata Corp LP) for all analyses. A p-value < 0.05 (two-tailed) was regarded as statistically significant.

4.3 Results

4.3.1 Participants and Groups

413 participants (mean age 63.2 years, 50% women) with symptomatic knee osteoarthritis and low vitamin D levels were enrolled in the VIDEO study from June 2010 to December 2011. 340 (82.3%) participants completed the study with 25(OH)D measurements at month 3 and 24. At baseline, participants who did not complete the study were more likely to be female and had lower tibial cartilage volume than those who completed, but there were no other significant differences in baseline characteristics between these groups[150]. Baseline characteristics of participants in three vitamin D status groups are showed in Table 4.1. Forty-six participants were classified as consistently insufficient (mean age 62.6, 52.2% females), 68 as fluctuating (mean age 62.9, 55.9% females) and 226 as consistently sufficient (mean age 63.5, 43.4%

females) vitamin D groups. There were no significant differences in baseline characteristics among groups.

Table 4.1 Baseline Characteristics in Groups with Different Serum Vitamin D Status

	Consistently insufficient (N=46)	Fluctuating (N=68)	Consistently sufficient (N=226)	*P value*
Sex, Female, No. (%)	24 (52.2%)	38 (55.9%)	98 (43.4%)	0.15
Age, y	62.6 (8.0)	62.9 (6.1)	63.5 (7.2)	0.68
Body Mass Index	30.6 (4.6)	29.0 (4.5)	29.4 (4.9)	0.21
Cartilage Volume, cm^3				
Later Tibial	2.0 (0.7)	2.0 (0.6)	2.1 (0.7)	0.32
Medial Tibial	1.4 (0.5)	1.5 (0.5)	1.6 (0.5)	0.12
Total Tibial	3.4 (1.1)	3.5 (0.9)	3.7 (1.1)	0.15
Tibiofemoral Cartilage Defects, Scores (0-8)				
Lateral	4.4 (1.8)	4.4 (1.7)	4.3 (1.9)	0.80
Medial	5.2 (2.0)	4.9 (2.1)	4.7 (2.1)	0.29
Tibiofemoral Bone Marrow Lesions, Scores (0-18)				
Lateral	0.6 (1.0)	0.9 (1.4)	0.9 (1.4)	0.48
Medial	1.7 (2.7)	1.6 (2.6)	1.3 (2.2)	0.55
Effusion-Synovitis Volume, cm^3	5.7 (5.6)	8.0 (9.5)	8.4 (8.5)	0.14
WOMAC Score System				
Pain (0-500)	144.2 (99.0)	130.0 (77.4)	133.8 (86.6)	0.91

Stiffness (0-200)	66.9 (44.8)	61.5 (39.1)	59.6 (39.4)	0.64
Function (0-1700)	524.2 (307.0)	440.7 (269.1)	461.8 (308.5)	0.37
Nonsteroidal anti-inflammatory drugs use, No. (%)	13 (28.3%)	19 (27.9%)	70 (31.0%)	0.86

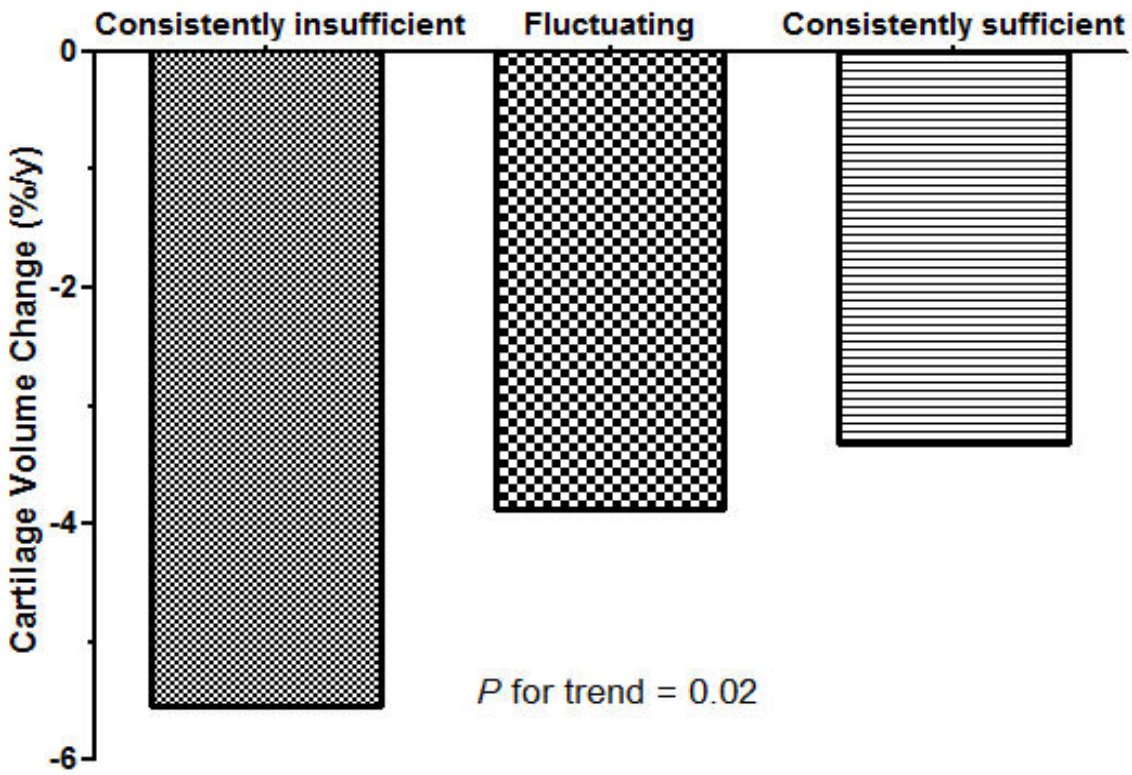
Values are mean (SD) unless otherwise stated.

One-way ANOVA or Kruskal-Wallis rank test was used for the comparisons.

4.3.2 Change in knee joint structures

There was a dose-response relationship between the status of serum vitamin D and change in total tibial cartilage volume (Figure 4.1). Participants with consistently sufficient vitamin D experienced significantly less loss of total tibial cartilage volume per year than participants with consistently insufficient vitamin D (Table 4.2, Figure 4.1). The differences between these two groups and among three groups remained significant after adjustment for age, sex, body mass index and change in season of blood sampling. A similar pattern was seen for medial and lateral tibial cartilage volume but the trend did not reach statistical significance in adjusted analyses (all $P \leq 0.10$) (data not shown). Similarly, participants with consistently sufficient vitamin D had significantly less increases in effusion-synovitis volume (absolute and percentage per year) compared with participants with consistently insufficient vitamin D (Table 4.3, Figure 4.2). The differences between these two groups and among three groups remained significant after adjustment for age, sex, body mass index and season of blood sampling (Table 4.3). In contrast, there were no significant differences in change in cartilage volume or effusion-synovitis volume between the fluctuating and consistently insufficient vitamin D groups. Additionally, there were no significant differences in changes in total cartilage defects and bone marrow lesions between and among groups (Table 4.2 and Table 4.3). We further adjusted for use of nonsteroidal anti-inflammatory drugs and found that the results remained largely unchanged (data not shown).

Figure 4.1 Tibial Cartilage Volume Change (%/y) in knee OA patients with or without sufficient serum vitamin D levels over 24 months



Consistently insufficient vitamin D group: serum 25-OHD≤ 50nmol/l at both month 3 and 24;

Fluctuating vitamin D group: serum 25-OHD> 50nmol/l at either time point;

Consistently sufficient vitamin D group: serum 25-OHD> 50nmol/l at both month 3 and 24.

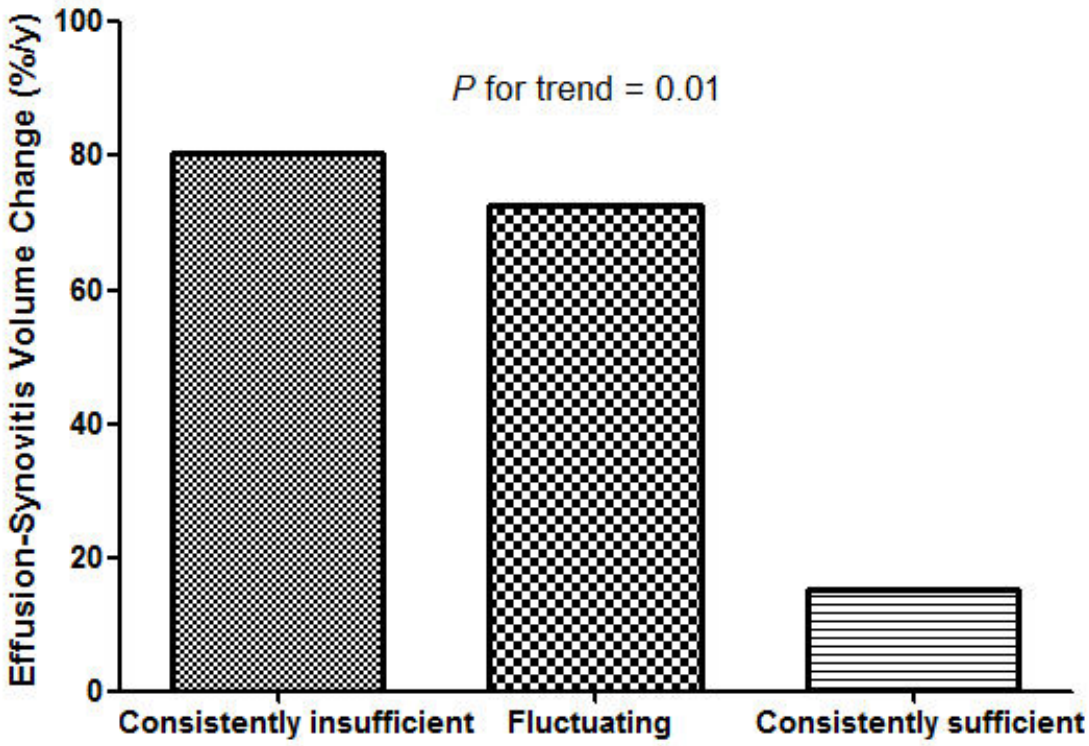
Table 4.2 Associations between maintaining vitamin D sufficiency and changes in cartilage volume and cartilage defects over 24 months

	Univariable		Multivariable*	
	β (95%CI)	P Value	β (95%CI)	P Value
Total tibial Cartilage Volume Change (%/y)				
Consistently insufficient	*Reference*		*Reference*	
Fluctuating	1.7 (-0.3, 3.6)	0.10	1.5 (-0.5, 3.5)	0.15
Consistently sufficient	2.2 (0.4, 4.0)	0.02	2.1 (0.3, 3.9)	0.03
P for trend		0.02		0.02
Change in Total Tibiofemoral Cartilage Defects				
Consistently insufficient	*Reference*		*Reference*	
Fluctuating	-0.3 (-0.8, 0.3)	0.40	-0.2 (-0.8, 0.4)	0.42
Consistently sufficient	-0.4 (-0.9, 0.1)	0.16	-0.4 (-0.9, 0.1)	0.15
P for trend		0.16		0.15

The dependent variables are percentage change in cartilage volume per year or absolute change in cartilage defects over 24 months;

*Adjusted for age, sex and body mass index and change in season of blood sampling.

Figure 4.2 Effusion-Synovitis Volume Change (%/y) in knee OA patients with or without sufficient serum vitamin D levels over 24 months



Consistently insufficient vitamin D group: serum 25-OHD≤ 50nmol/l at both month 3 and 24;

Fluctuating vitamin D group: serum 25-OHD> 50nmol/l at either time point;

Consistently sufficient vitamin D group: serum 25-OHD> 50nmol/l at both month 3 and 24.

Table 4.3 Association between maintain vitamin D sufficiency and change bone marrow lesions and effusion-synovitis volume over 24 months

	Univariable		Multivariable*	
	β (95%CI)	P Value	β (95%CI)	P Value
Change in Total Tibiofemoral bone marrow lesions				
Consistently insufficient	*Reference*		*Reference*	
Fluctuating	0.6 (-0.5, 1.6)	0.28	0.6 (-0.5, 1.6)	0.30
Consistently sufficient	0.5 (-0.4, 1.4)	0.25	0.5 (-0.4, 1.4)	0.25
P for trend		0.36		0.33
Effusion-Synovitis absolute Volume Change (ml)				
Consistently insufficient	*Reference*		*Reference*	
Fluctuating	0.5 (-2.8, 3.8)	0.77	0.7 (-2.5, 3.9)	0.66
Consistently sufficient	-2.4 (-4.5, -0.2)	0.03	-2.5 (-4.7, -0.2)	0.03
P for trend		0.01		<0.01
Effusion-Synovitis volume Change (%/y)				
Consistently insufficient	*Reference*		*Reference*	
Fluctuating	-9.5 (-118.4, 99.3)	0.86	2.2 (-112.2, 116.6)	0.97
Consistently sufficient	-69.5 (-133.4, -5.6)	0.03	-61.8 (-121.9, -1.7)	0.04
P for trend		0.01		0.01

The dependent variables are absolute change in bone marrow lesions or percentage change per year/absolute change in effusion-synovitis volume over 24 months;

* Adjusted for age, sex and body mass index and change in season of blood sampling.

4.3.3 Changes in knee symptoms

Changes in WOMAC scores over 24 months are shown in Table 4. There were significant differences in WOMAC physical function between consistently sufficient and consistently insufficient vitamin D groups in the mixed-effect models, adjusted for age, sex, body mass index and change in season of blood sampling (Table 4.4). Physical function improved time-dependently in consistently sufficient vitamin D group while fluctuated in other two groups over 24 months (Figure 4.3). The differences in total WOMAC score and physical function were significant among three groups (Table 4.4). There were no significant differences in WOMAC pain and stiffness between or among groups (Table 4.4). After further adjustment for nonsteroidal anti-inflammatory drugs use, the results remained largely unchanged (data not shown).

Table 4.4 Associations between maintaining vitamin D sufficiency and changes in clinical symptoms over 24 months

	Change Mean (95%CI)	Multivariable* β (95%CI)	P Value
Total WOMAC Score			
Consistently insufficient	-123.2 (-236.1, -10.4)	*Reference*	
Fluctuating	-111.4 (-194.8, -28.0)	11.8 (-128.5, 152.1)	0.87
Consistently sufficient	-240.4 (-298.4, -182.5)	-117.2 (-244.1, 9.6)	0.07
P for trend			<0.01
Pain			
Consistently insufficient	-34.7 (-66.6, -3.0)	*Reference*	
Fluctuating	-31.7 (-54.7, -8.8)	3.0 (-36.2, 42.3)	0.88
Consistently sufficient	-50.2 (-63.7, -36.6)	-15.4 (-49.9, 19.2)	0.38
P for trend			0.11

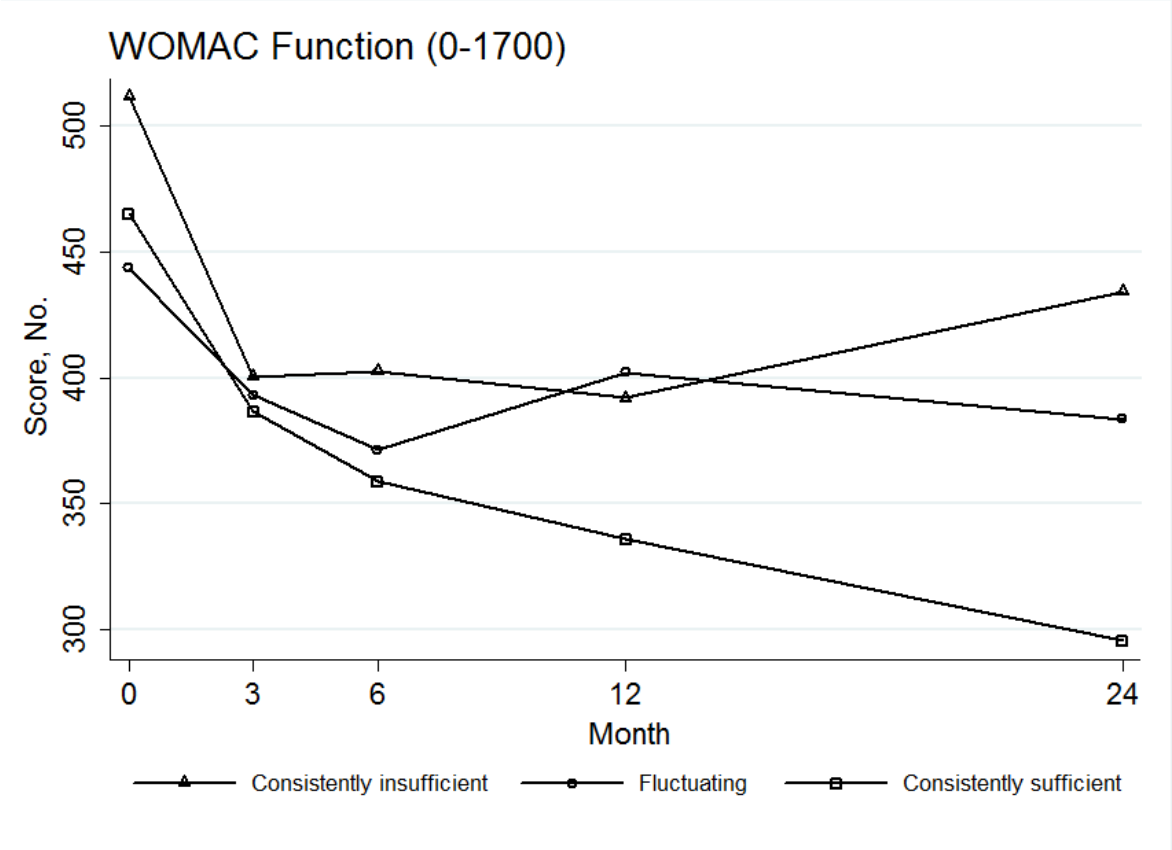
Stiffness			
Consistently insufficient	-11.7 (-25.1, 1.6)	*Reference*	
Fluctuating	-16.5 (-27.1, -6.0)	-4.7 (-21.7, 12.3)	0.59
Consistently sufficient	-20.3 (-26.4, -14.2)	-8.5 (-23.2, 6.1)	0.25
P for trend			0.17
Function			
Consistently insufficient	-76.0 (-153.6, 1.6)	*Reference*	
Fluctuating	-61.9 (-118.5, -5.3)	14.8 (-82.6, 112.2)	*0.77*
Consistently sufficient	-170.8 (-212.8, -128.8)	*-94.2 (-183.8, -4.5)*	*0.04*
P for trend			<0.01

WOMAC: Western Ontario McMaster Osteoarthritis Index; VAS: Visual Analog Scale;

Mixed effect model adjusted for age, sex, body mass index and change in season of blood sampling;

Change in WOMAC scores results are generated from mixed models adjusted for age, sex and body mass index and change in season of blood sampling.

Figure 4.3 Changes in WOMAC Function in knee OA patients with or without sufficient serum vitamin D levels over 24 months



Consistently insufficient vitamin D group: serum 25-OHD≤ 50nmol/l at both month 3 and 24;

Fluctuating vitamin D group: serum 25-OHD> 50nmol/l at either time point;

Consistently sufficient vitamin D group: serum 25-OHD> 50nmol/l at both month 3 and 24.

4.4 Discussion

To the best of our knowledge, this study is the first describing the differences in disease progression and symptoms among people with knee osteoarthritis by vitamin D status over time. It demonstrated that participants who maintained sufficient serum 25(OH)D levels over two years had decreased loss of tibial cartilage volume and less increase in effusion-synovitis volume comparing with those who did not. In addition, WOMAC physical function in

participants with persistent vitamin D sufficiency improved significantly more than those with persistent vitamin D insufficiency. However, we did not find significant differences in changes in cartilage defects, bone marrow lesions or knee pain between groups.

Results from previous randomised controlled trials have been mixed and do not provide consistent results[146 148-150]. McAlindon et al reported no effect of vitamin D supplementation (vitamin D3 2000 IU/day over two years, n=146) on cartilage volume loss or knee pain in patients with knee osteoarthritis[146]. However, the major limitations of this study were small sample size, the inclusion of participants with both vitamin D sufficiency and insufficiency, and the inclusion of participants with severe disease[147]. Participants with sufficient vitamin D may not benefit from vitamin D supplementation and patients with severe disease are unlikely to respond to any treatment[147]. Furthermore, a recent trial reported that vitamin D supplementation for three years (800IU per day for three years, n=474) did not slow progression of joint space narrowing (JSN) or reduce WOMAC pain, stiffness and function in knee osteoarthritis [148]. However, the researchers used a radiographic measurement as the outcome, which is less sensitive for change. In contrast, another randomised controlled trial reported that vitamin D supplement at a high dose (60,000 IU per day for 10 days followed by 60,000 IU once a month for a year, n=106) reduced knee pain and improved function in knee osteoarthritis, but this study was limited by its small sample size, short follow-up period and not examining structural change[149]. The VIDEO study aimed to overcome some of the limitations of previous studies but still had consistent negative results for the primary outcomes. In the secondary analyses, we found that the intervention group had small but statistically significant improvements in VAS knee pain and WOMAC function when compared with the placebo group[150]. In addition, 62% participants achieved a higher level of 25(OH)D (>50 nmol/l) at month 24 in the placebo group (unpublished data), which was thought in part to result from changes in lifestyle (e.g., sun exposure), dietary supplementation, supplementation of vitamin D outside the trial and seasonal variation.

We hypothesised that the high proportion of sufficient vitamin D level in the placebo group might dilute a beneficial effect of vitamin D supplementation. Thus we performed a post-hoc analysis to examine if maintaining sufficient vitamin D levels over time was associated with beneficial effects on joint structural and symptomatic changes in knee osteoarthritis patients in

VIDEO study. Those patients who maintained sufficient serum vitamin D levels over two years had less loss of total tibial cartilage volume per year than those who did not. Furthermore, there was a dose-response relationship between the status of serum vitamin D (consistently insufficient, fluctuating and consistently sufficient) and change in total tibial cartilage volume. In contrast, changes in cartilage defects and bone marrow lesions were not significantly different between and among groups. These results were largely consistent with the findings from two high-quality cohort studies[222] [223]. Felson et al[223] reported that low 25(OH)D status was not associated with change in cartilage defects in knee osteoarthritis patients while we reported that high serum 25(OH)D levels were associated with decreased knee cartilage volume loss over 2.7 years in older adults[222].

The relationship between vitamin D status and joint effusion-synovitis in knee osteoarthritis patients is unknown. In the VIDEO study, we recently reported that vitamin D supplementation significantly reduced the increase in effusion-synovit volume compared with placebo in patients with knee osteoarthritis particularly those with baseline effusion-synovitis[224]. The results from this study were consistent with this. The current study found that persistent vitamin D sufficiency was associated with improvement in physical function and total WOMAC score, but not with WOMAC pain and WOMAC stiffness. Again, these results were largely consistent with the findings from the VIDEO trial [150].

Overall, our results suggest that maintaining sufficient serum vitamin D may have a small but beneficial effect on retarding cartilage loss, reducing joint inflammation and improving physical function in knee osteoarthritis patients. The analyses in the current study have enabled us to use the actual serum vitamin D levels to define groups, which can reduce the potential confounding effect from those who achieved sufficient vitamin D levels in the placebo group during the trial. However, these data were post hoc and data-driven and need to be confirmed by further clinical trials. Further, loss of follow-up bias would exist; however, the retention rate in this trial was high (82%), and we used inverse probability weighting to count the impact of the missing values. In addition, we defined consistent vitamin D sufficiency or deficiency using 25(OH)D levels at only month 3 and 24, but the course of 25(OH)D levels between these measurements were unknown.

In conclusion, this post hoc analysis suggests beneficial effects of maintaining vitamin D sufficiency on cartilage loss, effusion-synovitis and physical function in people with symptomatic knee osteoarthritis.

Chapter 5 Vitamin D and inflammation in OA

This manuscript has been published (Zheng *et al,* British Journal of Nutrition 2018; 120: 41-48). The typeset version of the manuscript as it appeared in the journal is in Appendix II. The text of this chapter is the same as the published version, except where changes have been requested by the examiners. Thus, there are some repetitions of the methods.

5.1 Introduction

Osteoarthritis (OA) is a common chronic joint disease, associated with increased morbidity and disability risk and contributing to an enormous financial burden on health care systems worldwide[210]. In recent years, the pathophysiologic concept of OA has been changed from a degenerative joint disorder to a more complex concept involving multiple aetiologies and pathogeneses[225]. Inflammation is intricately linked to the etiology of OA and has been implicated in the pathogenesis of OA[226]. Experimental and observational studies have demonstrated that inflammatory and/or metabolic biomarkers are mediators of the inflammatory process of OA[227]. In addition, there is increasing evidence for a potential role of vitamin D deficiency in OA. Vitamin D receptor (VDR) is expressed in chondrocytes, osteoclasts and osteoblasts, and vitamin D can reduce bone turnover and cartilage degradation, and thus has potential to delay the development and progression of OA[30 121].

Interestingly, several experimental studies have reported that vitamin D may reduce the inflammatory response by modulating human monocyte function or VDR signalling[136 228]. Observational studies have shown that vitamin D deficiency is associated with increased inflammation in chronic conditions, including asthma, inflammatory bowel disease and rheumatoid arthritis[135 229]. Such evidence suggests that increased inflammation may be a key underlying mechanism linking vitamin D deficiency to OA, and vitamin D could modify OA disease progression through inhibition of inflammation. Previous studies have examined the effect of vitamin D supplementation on inflammatory biomarkers in older adults and patients with some chronic diseases, and have shown inconsistent results[230-233], but there has no study being reported whether vitamin D supplementation has effects on inflammatory and metabolic biomarkers in OA patients.

Recently we reported that compared with placebo, vitamin D supplementation had no significant effects on MRI-measured tibial cartilage volume or the Western Ontario and McMaster Universities Arthritis Index (WOMAC) assessed knee pain[150], but significantly reduced MRI-measured joint effusion-synovitis in patients with symptomatic knee OA[151]. This suggests that vitamin D supplementation could have anti-inflammatory effects by regulating serum levels of inflammatory or metabolic biomarkers in knee OA patients. The aim of the current study was, therefore, to determine whether vitamin D supplementation affected serum

inflammatory and metabolic biomarkers and whether variation in vitamin D status over two years was associated with change in biomarkers in patients with knee OA and vitamin D deficiency.

5.2 Materials and Methods

5.2.1 Study design

This study was a post-hoc analysis of the Vitamin D Effect on Osteoarthritis (VIDEO) study, which was a multicenter randomized, double-blind, placebo-controlled trial in knee OA patients with vitamin D deficiency. The method and protocol of the trial were described previously[175]. The trial was conducted from June 2010 to December 2013.

5.2.2 Participants

Briefly, eligible participants who had knee symptomatic OA (assessed using American College of Rheumatology, ACR criteria)[18] at least for 6 months and had pain of >20 mm on a 100-mm visual analog scale (VAS) with low levels of 25-hydroxyvitamin D [25(OH) D, between 12.5 and 60 nmol/L] were enrolled in Tasmania and Victoria, Australia. Participants with the following conditions were excluded: severe radiographic changes (grade 3 of Altman and Gold Atlas), severe knee pain on standing (> 80 mm on a 100-mm VAS), contraindications to magnetic resonance imaging (MRI), rheumatoid arthritis (RA) or psoriatic arthritis, lupus, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, conditions affecting oral drug absorption, anticipated knee or hip surgery within the next 2 years, history of significant trauma of knees (e.g. arthroscopy or injury to ligaments or menisci within one year preceding the study) and history of taking vitamin D or other investigational drugs, like some compound drugs including vitamin D affected serum vitamin D levels, within the last 30 days[175].

After the trial had completed, 200 participants were randomly selected for the measurements of inflammatory and metabolic biomarkers from Tasmania.

5.2.3 Ethics

Ethics approvals was received from Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616).

5.2.4 Randomization and treatment

Participants were allocated to either vitamin D or placebo arm at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a centrally automated allocation procedure with security in place to ensure allocation data cannot be accessed or influenced by any person from the investigative team. Participants received oral vitamin D capsules at a dose of 50,000 IU vitamin D3 (cholecalciferol) per month for 24 months in the treatment group. Participants received an identical inert placebo capsule in the placebo group[175].

5.2.5 Serum inflammatory and metabolic biomarkers levels measurement

All fasting blood samples were collected at baseline and month 24. The measurements were performed according to the manufacturer's instruction. Serum levels of high-sensitive C-reactive protein (hs-CRP), IL-6, IL-8, IL-10, resistin, leptin, adiponectin, adipsin and apelin-36 were measured. Serum hs-CRP was measured by enzyme immunoassays (IBL Inc.). Serum leptin and apelin-36 were measured by enzyme-linked immunosorbent assay (ELISA, Phoenix Pharmaceuticals Inc.). Serum adiponectin, adipsin, resistin were measured by ELISA (Millipore Inc.). Serum IL-6, IL-8, and IL-10 were measured by Bio-plex Luminex assay kits (Bio-Rad Laboratories Inc.). The inter-assay and intra-assay CVs were <10% and < 15% for all inflammatory biomarkers.

5.2.6 Serum vitamin D level measurement

Serum 25(OH)D was measured at baseline, month 3 and 24 using direct competitive chemiluminescent immunoassays (DiaSorin Inc.). The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%, respectively[150]. In addition, the season of blood sample was recorded. In this study, we defined serum 25(OH)D below than 50nmol/l as vitamin D deficiency.

5.2.7 Variation in vitamin D status

Participants were classified into two groups according to the levels of 25(OH)D at month 3 and 24 as follows: not consistently sufficient (serum 25(OH)D≤50nmol/l at either month 3 or 24), and consistently sufficient (serum 25(OH)D>50nmol/l at both month 3 and 24).

5.2.8 Assessment of effusion-synovitis and cartilage volume

MRI scans of the study knee were obtained according to a standardized protocol using a 1.5 T whole-body MRI unit with a commercial transmit-receive extremity coil at baseline and two years.

Effusion-synovitis assessed using T2-weighted/proton density–weighted fast spin echo (FSE) sequences at four regions (suprapatellar pouch, central portion, posterior femoral recess and subpoploteal recess). Effusion-synovitis in each subregion was scored individually according to Whole-Organ Magnetic Resonance Imaging Score (WORMS), grading collectively from 0 to 3 in terms of the estimated maximal distention of the synovial cavity: 0 refers to normal; 1 to <33% of maximum potential distention; 2 to 33%–66% of maximum potential distention; 3 to >66% of maximum potential distention. The presence of effusion-synovitis of the whole joint was defined as a score of ≥2 in any subregion[151].

Effusion-synovitis volume at 4 regions were isolated from the total volume selecting each region of interest (ROI) according to the intra-articular fluid-equivalent signal on a section-by-section basis and then resampled by means of bilinear and cubic interpolation for final 3D rendering using OsiriX imaging software (32-bit version 5.9, Pixmeo SARL)[234]. The intra-class correlation coefficient were from 0.96 to 0.97[235].

Cartilage volume was determined using the previously described image processing techniques[175]. The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled using bilinear and cubic interpolation for final three-dimensional rendering using OsiriX imaging software. The coefficient of variation was 2.1% to 2.2%[235]. Total tibial cartilage volume was calculated as the sum of the medial tibia and

lateral tibial cartilage plates. Change in cartilage volume and effusion-synovitis volume were calculated as follows:

$$\text{Absolute change (ml)} = Follow\ up\ volume - Baseline\ volume$$

5.2.9 Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes removed) using a stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Leicester, UK). Weight was measured to the nearest 0.1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307, Heine, New Hampshire, USA). Body mass index (BMI, in kg/m^2) was calculated.

5.2.10 Data analyses

Very few studies have examined the effect of vitamin D supplementation on inflammatory biomarkers; therefore, there was limited information to inform our sample size calculation. Based on a systematic review, the absolute difference in serum hs-CRP between people with OA and healthy controls is estimated as 1.19mg/L[236]. Data from the Tasmanian Older Adult Cohort Study provided a standard deviation (SD) estimate of 2.78mg/L. With these estimates, a sample size of 87 in each group would give 80% power with a 5% probability of type 1 error (α= 0.05, β= 0.8)[179 237] to detect this effect size for hs-CRP.

Baseline characteristic differences between the vitamin D supplementation and placebo groups were compared using Student's t-tests or Chi-square tests as appropriate. Box-cox transformation was used, when variables were not normally distributed, and transformed variables were used in the following analyses. The differences in changes of inflammatory biomarkers between treatment and placebo groups were analyzed using linear mixed effects model with adjustment for age, sex, BMI and seasonal change of blood sampling. The within-subject correlation between the repeated measures, including baseline and follow-up data, was taken into account using the individual participant identification as a random effect. The effect of vitamin D supplementation on biomarkers was evaluated by the interaction between treatment and time (i.e., month). The differences in changes of inflammatory biomarkers between not consistently sufficient and consistently sufficient groups were analyzed using linear mixed effects model with adjustment for age, sex, BMI and seasonal change of blood

sampling. Subgroup analyses were performed in participants with or without effusion-synovitis at baseline. We also have taken the weight change as confounder into analyses to make sure that the weight change did not play a role in this study. Further adjustment for changes in cartilage volume and effusion-synovitis volume were performed to account the effect of disease progression on serum biomarkers change. We used Stata 12.0 for Windows (Stata Corp LP) for all analyses. A p-value < 0.05 (two-tailed) was regarded as statistically significant.

5.3 Results

5.3.1 Baseline characteristics of participants

A total of 599 participants were screened for eligibility, 413 participants were enrolled and randomly assigned to vitamin D or placebo group (261 participants in Hobart and 152 participants in Melbourne), and 340 participants (82.0% retention rate in Hobart and 83.6% retention rate in Melbourne) completed the study (Figure 5.1). A total of 200 participants from Hobart was randomly selected for the inflammatory biomarkers' measurements, 94 participants from the placebo group and 106 from vitamin D treatment group.

The mean age of participants was 63.1 years, 107 (53.5%) were women and mean BMI was 29.5 kg/m^2. There were no significant differences in baseline characteristics (age, sex, BMI, serum 25(OH)D level, serum inflammatory biomarkers levels and season of blood sample) between the placebo and vitamin D groups (Table 5.1). There were no significant differences in baseline characteristics between those included and not included in this study (data not shown). Significant differences in baseline characteristics, inflammatory and metabolic biomarkers between consistently sufficient and not consistently sufficient groups were not found (data not shown).

Figure 5.1 Flowchart of the study

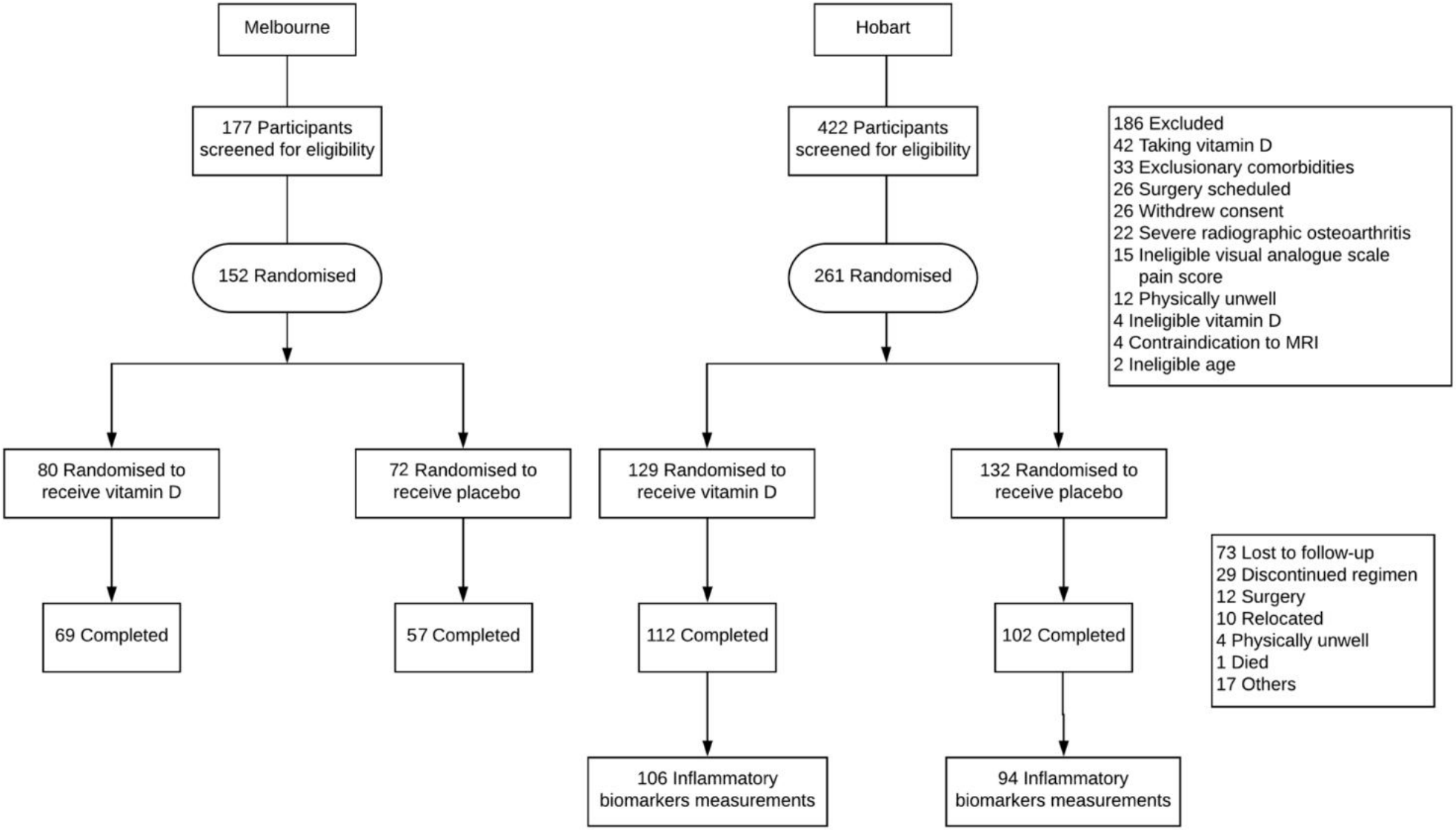


Table 5.1 Baseline characteristics of participants

	Vitamin D Group (N= 106)	Placebo Group (N= 94)	P value
Age, years	63.3 (7.5)	62.8 (7.3)	0.60
Women, No. (%)	53 (50.0%)	54 (57.4%)	0.29
BMI, kg/m^2	29.4 (7.5)	29.6 (4.0)	0.80
Serum 25(OH)D (nmol/L)	42.5 (11.7)	43.5 (12.6)	0.57
Serum biomarker			
*hs-CRP (ug/ml)	1.5 (0.8, 2 2.6)	1.3 (0.7, 2.5)	0.62
*IL-6 (pg/ml)	1.4 (0.4, 3.8)	1.2 (0.4, 3.7)	0.81
*IL-8 (pg/ml)	7.8 (5.7, 10.4)	7.6 (6.1, 10.9)	0.98
*IL-10 (pg/ml)	0.9 (0.3, 5.2)	0.6 (0.3, 3.5)	0.56
Resistin (pg/ml)	38.4 (14.9)	39.3 (13.2)	0.32
*Leptin (ng/ml)	19.2 (9.4, 58.1)	23.6 (9.7, 44.1)	0.90
*Adiponectin (ng/ml)	32.9 (18.2, 50.3)	26.5 (15.5, 43.8)	0.11
Adipsin (ng/ml)	4.0 (1.5)	3.9 (1.2)	0.84
Apelin (ng/ml)	1.0 (0.3)	1.0 (0.4)	0.84

Values are mean (standard deviation) or percentage unless otherwise stated.

* Skewed distribution, values are median (interquartile range).

BMI was calculated as weight in kilograms divided by height in meters squared. Student's t-tests or Chi-square tests.

Abbreviations: BMI, body mass index; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10.

5.3.2 Vitamin D supplementation and inflammatory and metabolic biomarkers

The mean serum 25(OH)D level increased significantly in the vitamin D treatment group (44.9 nmol/L) compared to the placebo group (7.0 nmol/L) over two years. The effect of vitamin D treatment on cytokines and adipokines is shown in Table 5.2. Vitamin D supplementation had no significant effect on change in serum inflammatory and metabolic biomarkers. There were no statistically significant differences in changes in any biomarkers between the placebo and vitamin D groups. After further adjustment for potential confounders including the seasonal change of blood sample, the differences between groups remained non-significant in the mixed effect model (Table 5.2). Within the group, serum resistin increased by 1.9 pg/ml (95% CI: 0.1, 3.8) in the placebo group and by 4.4 pg/ml (95% CI: 2.7, 6.2) in the vitamin D group from baseline to month 24. Difference in change in serum resistin between two groups was of borderline statistically significance (P= 0.05). Serum adipsin increased by 0.2 ng/ml (95% CI: 0.1, 0.4) in the vitamin D group, but did not increase in the placebo group from baseline to month 24. Serum hs-CRP, IL-6, IL-8, IL-10, leptin, adiponectin and apelin did not change significantly over 24 months in either group.

Further analyses were performed in participants who had baseline effusion-synovitis or not. Results remained largely unchanged (data not shown). The results remained unchanged after further adjustment for change in weight, and changes in cartilage volume and effusion-synovitis volume (data not shown).

Table 5.2 Comparisons of change in inflammatory biomarkers between vitamin D and placebo groups over 24 months

	Vitamin D group Change (N= 106), Mean (95% CI)[a]	Placebo group Change (N= 94), Mean (95% CI)[a]	Between-Group Difference, Mean (95% CI)[b]	P Value
Serum 25(OH)D (nmol/L)	45.0 (40.8, 49.0)	7.4 (3.0, 11.8)	37.5 (31.5, 43.6)	0.00
hs-CRP (ug/ml)	0.3 (-0.2, 0.7)	-0.0 (-0.5, 0.5)	0.3 (-0.4, 1.0)	0.43
IL-6 (pg/ml)	-2.3 (-5.6, 0.9)	-0.7 (-4.2, 2.8)	-1.6 (-6.4, 3.2)	0.51
IL-8 (pg/ml)	-3.1 (-6.5, 0.3)	-0.0 (-3.7, 3.6)	-3.1 (-8.1, 2.0)	0.24
IL-10 (pg/ml)	-2.0 (-9.3, 5.3)	-0.7 (-8.5, 7.1)	-1.3 (-12.0, 9.4)	0.81
Resistin (pg/ml)	4.4 (2.7, 6.2)	1.9 (0.1, 3.8)	2.5 (-0.0, 5.1)	0.05
Leptin (ng/ml)	-0.2 (-3.4, 3.0)	-0.8 (-4.2, 2.6)	0.6 (-4.0, 5.3)	0.79
Adiponectin (ng/ml) ¶	0.01 (-0.02, 0.04)	0.00 (-0.03, 0.03)	0.01 (-0.04, 0.06)	0.66
Adipsin (ng/ml)	0.2 (0.1, 0.4)	0.1 (-0.0, 0.3)	0.1 (-0.1, 0.3)	0.39
Apelin (ng/ml)	-0.1 (-0.1, 0.0)	0.0 (-0.1, 0.1)	-0.1 (-0.2, 0.0)	0.13

¶ box-cox transformation; [a] Change in inflammatory biomarkers are generated from mixed models adjusted for age, sex, BMI and change in season of blood sampling; [b] Between-group difference was calculated using vitamin D group values minus placebo group values. Vitamin D status and change in biomarkers

5.3.3 Vitamin status and change in biomarkers

There were no statistically significant differences in changes of these biomarkers between consistently sufficient and not consistently sufficient groups (Table 5.3). In the consistently vitamin D sufficient group, there were significant increases in serum resistin and adipsin (3.8 pg/ml and 0.3 ng/ml, respectively) and a significant decrease in serum IL-8 (3.0 pg/ml) from baseline to month 24 (Table 5.3). In contrast, there was no significant change over the study period in the not consistently sufficient group. Serum IL-6, IL-10, CRP, leptin, adiponectin, and apelin did not change significantly from baseline to months 24 in either group.

The results remained largely unchanged if patients with and without baseline effusion-synovitis were separated for analyses (data not shown). Results remained largely unchanged after further adjustment for changes in weight, cartilage volume and effusion-synovitis volume (data not shown). In addition, no significant associations were found between the changes in inflammatory/metabolic biomarkers and change in 25(OH)D over 24 months, and the results remained largely unchanged after further adjustment for the baseline serum 25(OH)D level (except for change in resistin, P= 0.04) (data not shown).

Table 5.3 Comparison of change in inflammatory biomarkers between different vitamin D status over 24 months

	Consistently sufficient Change (N= 139), Mean (95% CI)[a]	Not consistently sufficient Change (N= 61), Mean (95% CI)[a]	Between-group Difference, Mean (95% CI)[b]	P value
Serum 25(OH)D (nmol/L)	37.8 (34.1, 41.6)	3.1 (-2.6, 8.7)	34.8 (28.0, 41.6)	0.00
hs-CRP (ug/ml)	0.2 (-0.2, 0.7)	-0.1 (-0.7, 0.6)	0.3 (-0.5, 1.1)	0.46
IL-6 (pg/ml)	-2.8 (-5.7, 0.0)	1.3 (-3.0, 5.6)	-4.1 (-9.3, 1.0)	0.12
IL-8 (pg/ml)	-3.0 (-6.0,-0.0)	1.4 (-3.1, 6.0)	-4.5 (-9.9, 1.0)	0.11
IL-10 (pg/ml)	-3.3 (-9.6, 3.1)	2.9 (-6.8, 12.6)	-6.2 (17.8, 5.4)	0.29
Resistin (pg/ml)	3.8 (2.3, 5.4)	1.9 (-0.4, 4.3)	1.9 (-0.9, 4.7)	0.18
Leptin (ng/ml)	-0.1 (-2.9, 2.7)	-1.3 (-5.5, 2.9)	1.1 (-3.9, 6.2)	0.66
Adiponectin (ng/ml) ¶	0.02 (-0.01, 0.04)	-0.01 (-0.06, 0.03)	0.03 (-0.02, 0.08)	0.26
Adipsin (ng/ml)	0.3 (0.1, 0.4)	0.1 (-0.1, 0.3)	0.2 (-0.1, 0.4)	0.13
Apelin (ng/ml)	-0.0 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.72

¶ box-cox transformation; [a] Change in inflammatory biomarkers are generated from mixed models adjusted for vitamin D treatment, age, sex, BMI and change in season of blood sampling; [b] Between-group difference was calculated using consistently sufficient group values minus not consistently sufficient group values.

5.4 Discussion

To the best of our knowledge, this study is the first to explore the effect of vitamin D supplementation on inflammatory and metabolic biomarkers in patients with knee OA and to compare the effect of vitamin D sufficiency on levels of serum inflammatory and metabolic biomarkers in OA patients. Vitamin D supplementation had no significant effects on serum inflammatory and metabolic biomarkers in patients with knee OA. Furthermore, there were no significant differences in serum inflammatory and metabolic biomarkers between those who were consistently sufficient and those who were not over 24 months. These results suggest that vitamin D supplementation and maintaining sufficient vitamin D status may not have effects on systemic inflammation in knee OA patients.

Low-grade systemic inflammation triggered by abnormally inflammatory or metabolic biomarkers has been implicated in the OA pathogenesis. There is a vast amount of evidence linking inflammatory biomarkers and OA, as well as the association between vitamin D and inflammation. Serum hs-CRP levels were statistically significantly higher in OA group than control group and were associated with increased pain and decreased physical function[236]. Serum IL-6 was correlated with radiographic OA, knee cartilage loss and increased knee pain over time[238 239]. Adipokines such as leptin and resistin may disrupt cartilage homoeostasis through directly inducing joint structural degradation or regulating local inflammatory processes and are regarded as metabolic biomarkers in the inflammatory process of OA[240 241]. Serum leptin was associated with reduced knee cartilage volume and increased loss of cartilage thickness[242]. Furthermore, high serum leptin and IL-6 were associated with reduced 25(OH)D levels over time[243]. These suggest that systemic inflammation triggered by inflammatory or metabolic biomarkers may be a key underlying mechanism linking vitamin D deficiency to OA.

Although there has been no previous RCT examining the effect of vitamin D supplementation on inflammatory and metabolic biomarkers in knee OA patients, some RCTs have examined

the effect in healthy individuals, older adults, or patients with other chronic diseases such as obesity, asthma, diabetes and chronic kidney disease, and reported inconsistent results[244]. The inconsistency between these study findings may be caused by small sample sizes, diverse characteristics of participants, treatment with different doses, and measurements of different inflammatory and metabolic biomarkers for the different clinical trials[245]. For example, two RCTs with small sample size were conducted in patients with diabetes. One study reported that supplementation with 50,000IU vitamin D per two weeks for 12 weeks in 60 patients significantly reduced serum hs-CRP level compared to the placbo group[246], but another study reported that treatment with 50,000IU per week vitamin D and/or 1000mg calcium per day twice for 8 weeks did not result in significant difference in change in serum CRP and leptin, but did result in significant reduction in serum IL-6 and TNF-α comparing with the placebo group in 118 diabetic patients[231].

Our results are consistent with finding from RCTs in healthy or older adults without specific health conditions as vitamin D supplementation in these groups has shown no effect on inflammatory and metabolic biomarkers[247-250]. A randomised placebo-controlled trial was conducted in elderly adults without a specific disease in Australia, aiming to examine the effects of vitamin D supplementation for 12 months on hs-CRP, leptin, adiponectin, IL-6 and IL-10[251]. IL-6 was numerically higher in those participants supplementing with 1500 μg/month vitamin D comparing with 750 μg/month, although this was not significant. In our current study, we found that vitamin D supplementation had no significant effect on serum inflammatory and metabolic biomarkers, with the exception of a possible effect on serum resistin, in patients with knee OA. Serum resistin increased twofold more in the vitamin D group than in the placebo group, but the difference was of borderline significance. Although evidence shows that serum resistin is a pro-inflammatory cytokine and is positively associated with severity of OA[252], the clinical relevance of this finding is unknown. Subgroup analyses were performed in participants who had baseline effusion-synovitis or not and results remained unchanged. We previously reported that vitamin D supplementation relieved the progression of effusion-synovitis in patients with an inflammatory OA phenotype[151]. These indicated that vitamin D supplementation would have effects on local rather than systemic inflammation. The underlying mechanisms are unclear and need to be explored by further studies.

A high proportion of participants in the placebo group achieved sufficient vitamin D level in month 24 (62% participants >50nmol/L) in VIDEO study, which may have masked the effect of vitamin D supplementation on inflammatory and metabolic biomarkers. Therefore, we performed further post-hoc analyses to examine if maintaining sufficient vitamin D level over the treatment period had effects on the biomarkers. Although serum resistin and adipsin increased, and serum IL-8 decreased from baseline to months 24 in the consistently vitamin D sufficient group, changes in serum resistin, adipsin and IL-8 were not significantly different between the patients with different vitamin D status. Up to now, only one cross-sectional study compared the level of inflammatory cytokines (IL-1β, 2, 4, 5, 6, 8,10, 12,13 and hs-CRP) in different serum vitamin D status (deficient, insufficient or sufficient) in patients with knee OA, and did not find that vitamin D status was associated with circulating inflammatory biomarkers[253]. These results were generally consistent with the findings in our current study. Maintaining serum vitamin D sufficiency may not have effects on systemic inflammation in knee OA patients.

We used the direct competitive chemiluminescent immunoassays (Diasorin Liaison assay) to measure the serum 25(OH)D which may be not as sensitive and specific high performance liquid chromatography (HPLC) methodology; however, it showed good correlation with HPLC, and would also be specific for both 25(OH)D2 and 25(OH)D3[254 255]. The vitamin D status, measured via serum 25(OH)D concentrations, can be affected by factors such as obesity. In addition, expression of vitamin D dependent genes could be served as a marker of vitamin D status, and this may also influence the effect of vitamin D supplementation on inflammatory markers[256 257]. These need to be explored in future studies.

There were some limitations of the current study. First, it was a post-hoc analysis within a subsample of an RCT, which was not designed to examine the effect of vitamin D supplementation on inflammatory and metabolic biomarkers in patients with knee OA. The findings need to be confirmed by further RCTs using these biomarkers as the primary endpoints. Second, this study had reduced sample size than what was designed for the original study. However, the sample size is sufficient to detect a significant difference for hs-CRP. Third, there was a high proportion of participants in the placebo group who achieved sufficient vitamin D levels at month 3 and month 24 (62% participants >50nmol/L) in the RCT study, which could

have diluted the effect of vitamin D supplementation. Thus, we performed further post-hoc analyses using variations in vitamin D status over the treatment period and the results were consistent. Fourth, 3 time points (baseline, 3 months to 24 months) may not be adequate to define the "consistently or inconsistently vitamin D sufficient", and the inflammatory and metabolic markers were not measured at an intermediate time point. It was unknown if vitamin D supplementation had intermediate effects on inflammatory markers in knee OA patients. Therefore, further studies are required.

In conclusion, vitamin D supplementation and maintaining vitamin D sufficiency did not alter serum levels of inflammatory and metabolic biomarkers over 2 years in knee OA patients who were vitamin D insufficient, suggesting they may not affect systemic inflammation in knee OA patients.

Chapter 6 Effects of vitamin D on depressive symptoms

This manuscript has been published (Zheng *et al, Journal of the American Medical Directors Association* 2018). The typeset version of the manuscript as it appeared in the journal is in Appendix II. The text of this chapter is the same as the published version, except where changes have been requested by the examiners. Thus, there are some repetitions of the methods.

6.1 Introduction

Depression is a major global public-health problem and is projected to be the second leading cause of disease burden by the year 2030[258]. Due to population ageing, depression in later life is a common comorbidity of many chronic diseases[85]. Although depression is less prevalent among older than younger adults, it still has detrimental consequences[259]. Similarly, osteoarthritis (OA) is the most prevalent chronic joint disease and the leading cause of disability in individuals and has a substantial financial burden on the health system[6 24]. It is predicted that 130 million individuals of more than 60 years old will suffer from OA worldwide by 2050[260]. Depression and depressive symptoms are common among individuals with OA. Stubbs and colleagues reported that 19.9% of those with OA had depressive symptoms, with a relative risk of 1.17 in those with OA compared to those without[86]. Concomitant depression in OA patients contributes to its increased disease burden and troubles with disease management[261 262].

Vitamin D deficiency or insufficiency is a global public health problem and affects nearly a billion people worldwide[113]. Vitamin D deficiency is associated with a range of mental disorders including depression[113]. Vitamin D receptors (VDR) exist in the brain, and vitamin D can boost the levels of brain chemical monoamines, which is necessary for a positive mood and has possible neuroprotective roles that vitamin D may play through its effects on inflammation[153 263-265]. Furthermore, a positive association between vitamin D deficiency and depression has been reported in previous epidemiological and clinical studies in the general population including children, older adults and patients with other chronic diseases[159 266 267]; however, it remains unknown whether the relationship is causal[266]. Although the biological mechanisms underlying the associations between vitamin D deficiency and depression are unclear and may be complicated, the therapeutic potential of vitamin D supplementation for depression has been highlighted[159]. Thus far, randomized controlled trial (RCT) evidence examining whether vitamin D supplementation can improve depression has been investigated in the general population and shows inconsistent findings. The contradictory evidence may be caused by variations in baseline vitamin D levels, vitamin D dosages, sample sizes, duration of follow-up, outcome measurements and investigated populations[162]. A meta-analysis reviewed nine clinical trials and reported no significant reduction in depression after vitamin

D supplementation, but most of the studies focused on individuals with low levels of depression or who had sufficient vitamin D at baseline[164]. Further well-designed vitamin D supplementation RCTs among individuals who are both depressed and vitamin D deficient are needed.

Although depression is prevalent in OA patients and a positive association between vitamin D deficiency and depression has been demonstrated, no study has examined the effect of vitamin D supplementation on depressive symptoms in people with OA so far. This study primarily aims to determine the effect of vitamin D supplementation on depressive symptoms in patients with knee OA and vitamin D deficiency. An ancillary aim of this study is to determine whether maintaining sufficient serum vitamin D has beneficial effects on depressive symptoms[150].

6.2 Materials and Methods

6.2.1 Trial design and participants

This study is a pre-specified secondary analysis of the Vitamin D Effect on Osteoarthritis (VIDEO) study, which was conducted from June 2010 to December 2013. VIDEO was a multicenter, randomized, double-blind, placebo-controlled trial to determine the effect of vitamin D supplementation on knee structures and symptoms among patients with symptomatic knee OA. This secondary analysis aims to determine the effect of vitamin D supplementation on depressive symptoms in patients with knee OA and vitamin D deficiency. The ancillary aim of determining whether maintaining sufficient serum vitamin D has beneficial effects on depressive symptoms was added because there was a high proportion of participants having unforeseen improvements in serum 25-hydroxyvitamin D [25(OH)D] levels in the placebo group of our RCT as previously published[235].

The methods were described in the published protocol of the trial [175], and the results for primary outcomes have been published[150]. Participants who suffered from symptomatic knee OA, which was assessed using the American College of Rheumatology (ACR) criteria[18], for at least 6 months with knee pain >20 mm on a 100-mm visual analog scale (VAS) and serum levels of 25-hydroxyvitamin D [25(OH)D] between 12.5 and 60 nmol/L were enrolled in Tasmania and Victoria, Australia. Participants with grade 3 radiographic changes (Altman and Gold Atlas), severe knee pain on standing (>80 mm on a 100-mm VAS), other rheumatic diseases such as

rheumatoid arthritis, psoriatic arthritis and lupus, contraindication to MRI, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, anticipated knee or hip surgery within the next 2 years and history of taking vitamin D within the previous 1 month were excluded[175].

6.2.2 Ethics

All participants provided informed written consent and the study was approved by the Ethics Committee in Tasmania and Melbourne (reference number: H1040 and CF10/1182-2010000616, respectively).

6.2.3 Randomization and intervention

Participants were allocated to either the vitamin D or placebo arm at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a centrally automated allocation procedure with security in place to ensure allocation data could not be accessed or influenced by any person from the investigative team. Participants, research coordinators and investigators were all blinded to treatment assignment. Blinding was maintained until all data were collected, cleaned, confirmed for accuracy and analyses of primary outcomes were performed.

Participants received 50,000IU (1.25mg) oral vitamin D_3 capsule (cholecalciferol) monthly for 24 months or an identical inert placebo[175]. Both the vitamin D_3 compound and placebo were prepared by and purchased from Nationwide Compounding Pharmacy, Melbourne, Australia.

6.2.4 Patient health questionnaire-9

Depressive symptoms were assessed using the patient health questionnaire (PHQ-9) at baseline, month 3, 6, 12 and 24. PHQ-9 is a valid and reliable self-reported depression instrument, which is widely used in multipurpose diagnoses, severity measures in the clinic, as well as assessing depression outcomes in research[204 205]. It is a nine-item questionnaire with a score range of 0 to 27, with each item being scored from 0 to 3 (not at all, several days, more than half days and nearly every day). Using the mental health professional interview as the criterion standard, PHQ-9 scores of 5-9, 10-14, 15- 20 and >20 represent mild, moderate, moderately severe and

severe depression, respectively. A PHQ-9 scores ≥ 10 has a sensitivity of 88% and specificity of 88% for major depression[204].

6.2.5 Serum 25(OH)Dmeasurement

Serum 25(OH)D was measured at baseline, month 3 and 24 using direct competitive chemiluminescent immunoassays, which is an accurate and reproducible automated tool[182] (DiaSorin Inc.). The Intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%, respectively[150]. The season of blood sample was recorded.

6.2.6 Knee symptoms measurement

Knee symptoms were assessed at baseline and months 3, 6, 12, and 24 using the Western Ontario and McMaster Universities OA Index (WOMAC), which is a widely used instrument to evaluate the functional capacity in patients with OA and demonstrates high performance in clinical trials[183]. The Index contains 24 questions (5 related to pain, 2 to stiffness and 17 to physical function) with scores ranging from 0 (none) to 100 (severe) for each question. The total WOMAC score (0-2400) is the sum of subscale scores including pain (0-500), stiffness (0-200) and physical function (0-1700).

6.2.7 Physical activity

Physical activity (PA) was assessed by the international physical activity questionnaire (IPAQ), which has been developed and tested for use in adults (age range of 16-69 years). PA status (insufficiently active, sufficiently active and highly active) was calculated according to the scoring protocol available at http://www.ipaq.ik.se. The IPAQ instrument was valid and reliable for self-reports and monitoring population levels of physical activity among adults (18 to 65 years old) in diverse settings[268].

6.2.8 Anthropometrics and social demographic characteristics data

Height was measured to the nearest 0.1 cm (with shoes removed) using a stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Leicester, UK). Weight was measured to the nearest 0.1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307, Heine, New

Hampshire, USA). Body mass index (BMI, in kg/m^2) was calculated[175]. Obesity status were defined as the normal weight, overweight and obese depending the BMI according the criteria[269]. Patients filled out a questionnaire which collected information on education history (grade 0: less than high school, grade 1: high school degree and grade 2: superior than high school degree), current regular smoker (yes or no), current medical conditions and medication uses.

6.2.9 Statistical methods

The primary analysis compared the effect of vitamin D supplementation on depressive symptoms as measured by the PHQ-9 between the vitamin D group and the placebo group. Given the previously published high level of correction of deficiency in the placebo group (62% reached vitamin D sufficiency at month 24 in the placebo group), a secondary analysis was also performed by vitamin D status[150 270]. Participants were classified into two groups according to their serum 25(OH)D levels at month 3 and 24 as follows: not consistently sufficient (serum 25(OH)D≤50nmol/l at month 3 and/or 24), and consistently sufficient (serum 25(OH)D>50nmol/l at both month 3 and 24).

Based on *Lowe's* study[205], we anticipated a standard deviation (SD) of 5.4 for the change in PHQ-9. The sample size calculation assumed $\alpha = 0.05$ and $\beta = 0.20$ and was performed based on the Cohen formula[179]. With 400 participants, a difference between groups of 1.2 units on the score is detectable with 80% power.

Differences in baseline characteristics between vitamin D and placebo groups were compared using independent *t*-tests or Chi-square tests as appropriate. Repeated-measures mixed effect models were used to examine changes in PHQ-9 scores over 24 months in the vitamin D supplementation versus the placebo group and the group that maintained vitamin D sufficiency between month 3 and 24 versus the group that did not maintain vitamin D sufficiency. The models were adjusted for age, sex and BMI. Further adjustments for knee pain, joint function, baseline PHQ-9 score, serum 25(OH)D level, self-reported depression and/or anti-depressant medication were performed. Missing data due to loss to follow-up or nonresponses were accounted for in the multilevel mixed-effect model. Sub-group analysis was performed to examine whether the effect of vitamin D supplementation and maintaining vitamin D

sufficiency varied by the presence of depressive symptoms at baseline. A reduction of PHQ-9 score of greater than 2.59/27 was used as the cut-off for the minimal clinically important difference (MCID) based on the *Lowe's* recomendation which was developed for those with major depression[205].

All tests were two-sided and a *P* value <0.05 was considered as statistically significant. Stata version 12.0 was used to perform statistical analyses.

6.3 Results

6.3.1 Baseline characteristics of participants

Figure 6.1 shows the flow of study participants. Five hundred and ninety nine participants were screened for eligibility, and 413 participants were enrolled (Figure 6.1). 209 and 204 participants were randomly assigned to receive vitamin D and placebo, respectively. Over 24 months, 340 participants (intervention N= 181, placebo N= 159, 82.3% retention rate) completed the study. The mean age of participants was 63.2 (7.0) years, 208 (50.3%) were women and mean BMI was 29.6 (5.0) kg/m^2. The baseline prevalence of depression (PHQ-9 score ≥5) was 25.4% (mild and moderate to severe depression was 17.6% and 7.8%, respectively). The actual range of PHQ-9 score in this study was 0-24 in the vitamin D supplementation group and 0-22 in the placebo group.

Table 6.1 presents the characteristics of participants stratified by treatment groups. The two groups had similar demographic and social-demographic features, medication uses, physical activity, knee symptoms, and prevalence of depression. There were also no significant differences in baseline characteristics, physical activity, knee symptoms and prevalence of depression between the consistently sufficient and not consistently sufficient groups (data not shown); however, the baseline serum 25(OH)D level and PHQ-9 score was higher in the consistently sufficient group (45.1 versus 41.5 nmol/L, *P* value= 0.01; 3.3 versus 2.4, *P* value= 0.046). The characteristics of participants stratified by maintaining and not maintaining vitamin D sufficiency groups are presented in the Supplementary Table.

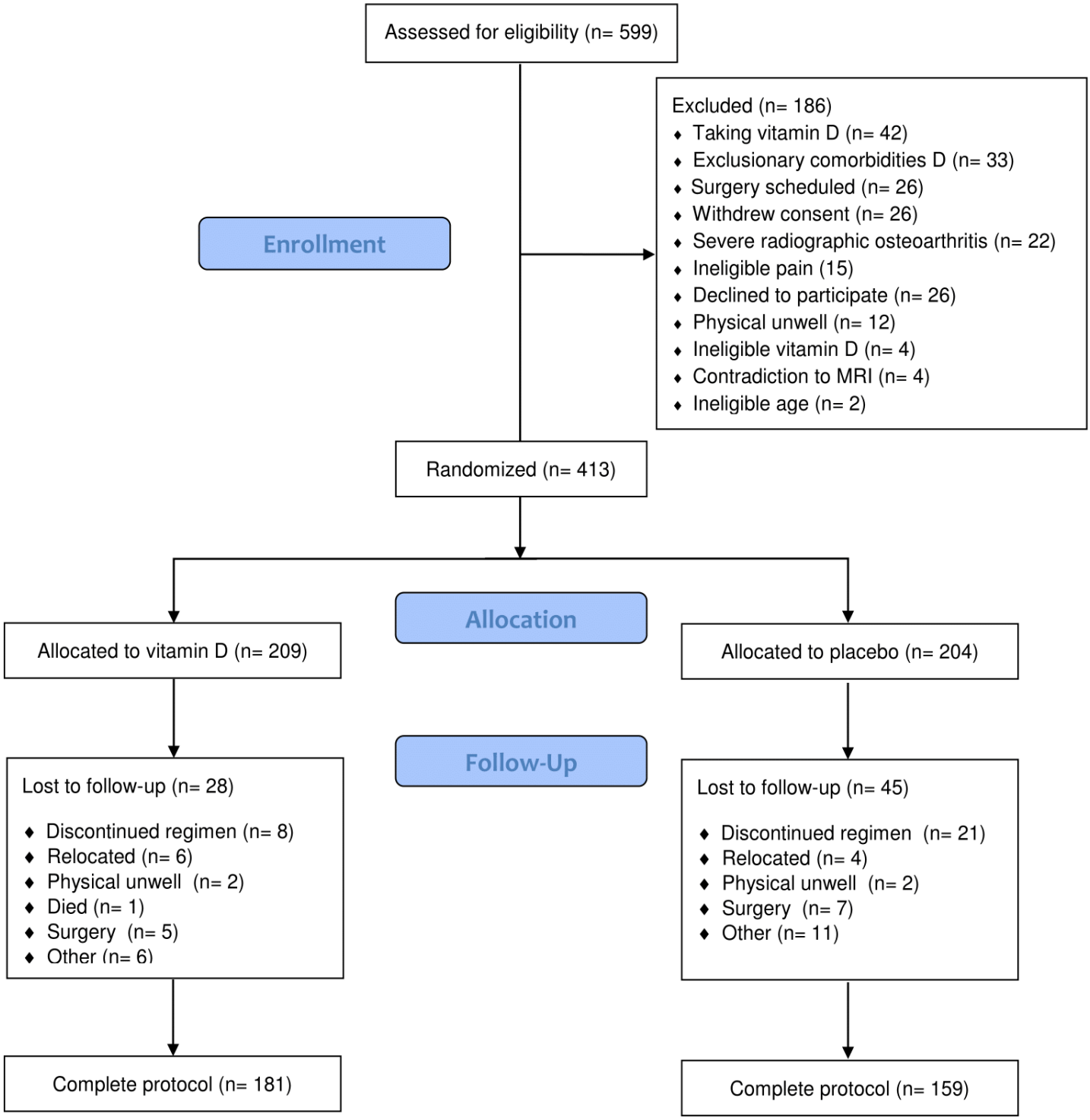


Figure 6.1 Flowchart of the study

Table 6.1 Baseline characteristics of participants between vitamin D supplementation and placebo groups

	Vitamin D Group (N= 209)	Placebo Group (N= 204)
Age, mean (SD), years	63.5 (6.9)	62.9 (7.2)
Female, No. (%)	106 (52.7)	102 (50.0)
Body mass index, mean (SD)	29.6 (5.4)	29.6 (4.6)
Normal weight, No. (%)	42 (20.1)	23 (11.3)
Overweight, No. (%)	88 (42.1)	90 (44.1)
Obese, No. (%)	79 (37.8)	91 (44.6)
Serum 25-(OH) D levels, mean (SD), nmol/L	43.7 (11.8)	43.8 (12.7)
Regular smokers, No. (%)	92 (44.7)	98 (48.5)
Self-reported depression, No. (%)	10 (4.8)	14 (6.9)
Anti-depressant medicine use, No. (%)	6 (3.0)	11 (5.6)
Physical activity		
Insufficiently active, No. (%)	39 (22.2)	37 (20.9)
Sufficiently active, No. (%)	111 (63.1)	114 (64.4)
Highly active, No. (%)	26 (14.8)	26 (14.7)
Highest education		
<High school, No. (%)	26 (12.9)	25 (12.1)
High school, No. (%)	73 (36.1)	78 (37.7)
>High school, No. (%)	103 (51.0)	104 (50.2)
WOMAC score		

Pain (0-500), mean (SD)	137.9 (88.8)	134.7 (83.4)
Function (0-1700), mean (SD)	487.9 (318.1)	467.6 (292.8)
Stiffness (0-200), mean (SD)	61.5 (41.5)	61.7 (40.1)
PHQ-9 score (0-27), mean (SD)	3.4 (4.1)	3.0 (4.0)
Depression status		
No depression, No. (%)	145 (72.1)	151 (77.0)
Mild depression, No. (%)	38 (18.9)	32 (16.3)
Moderate to severe depression, No. (%)	18 (9.0)	13 (6.6)

PHQ-9, patient health questionnaire depression scale.

No depression was defined as PHQ-9 scores of <5; Mild depression was defined as PHQ-9 scores 5-9; Moderate to severe depression was defined as PHQ-9 scores of ≥10.

Student t-test or chi2 test was used for the comparison.

6.3.2 Vitamin D supplementation, vitamin D status and change in depressive symptoms

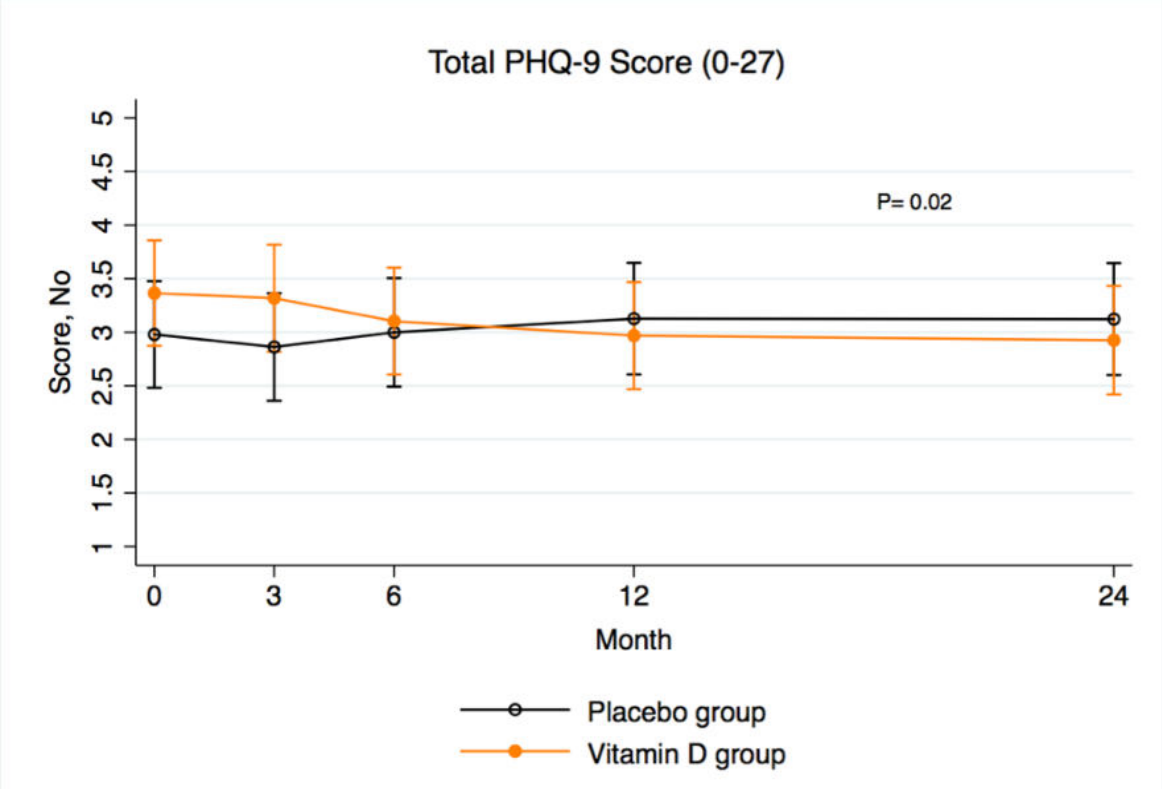
6.3.2.1 Whole sample

After 24 months, serum 25-(OH) D levels increased from 43.7 ± 11.8 nmol/l to 84.5 ± 17.3 nmol/l in the vitamin D group and increased from 43.8 ± 12.7 nmol/l to 50.6 ± 17.5 nmol/l in the placebo group, as described previously[150].

Table 6.2 and 6.3 describe the changes in PHQ-9 scores in vitamin D treatment groups and vitamin D status groups. PHQ-9 scores improved more in the vitamin D supplementation group compared to the placebo group (β: -0.66, 95% CI: -1.22 to -0.11, Figure 2) with adjustment for age, sex and BMI. The results remained significant with further adjustment for baseline PHQ-9 score, self-reported depression and usages of anti-depressant medication (β: -0.59, 95% CI: -1.15 to -0.04). Although the rate of obesity in the placebo group was higher than the vitamin D group, the results remained largely unchanged after adjustment of obesity (data not shown). PHQ-9 scores also improved more in those participants who maintained vitamin D sufficiency

between month 3 and 24 compared to those who did not maintain sufficiency (β: -0.77, 95% CI: -1.45 to -0.08, Table 6.3 and Figure 6.3) with adjustment for potential confounders, age, sex, BMI, serum 25(OH)D level, PHQ-9 score, self-reported depression and usages of anti-depressant medication at baseline.

Figure 6.2 Change in PHQ-9 scores in the vitamin D supplementation group and the placebo group



Vertical bars indicate 95% CIs for the mean scores.

P value was for the difference between the 2 groups in PHQ-9 score changes from baseline to month 24.

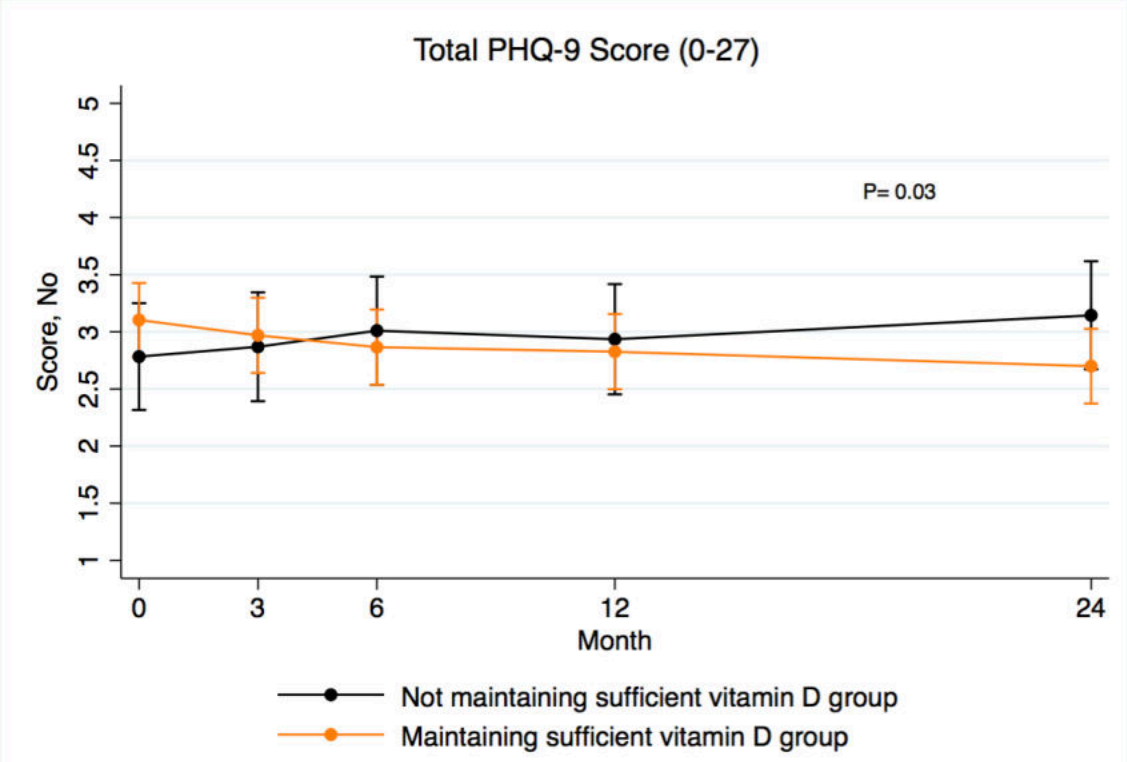
Table 6.2 Effects of vitamin D supplementation over 24 months on change in PHQ-9

	Baseline Mean (SD)	Month 24 Mean (SD)	Mean change Mean (95% CI)	Between-group difference change Mean (95% CI)	P value
Whole sample					
Placebo Group (N= 204)	3.0 (4.0)	3.2 (3.8)	0.21(-0.19 to 0.61)	-0.66 (-1.22 to -0.11)	0.02
Vitamin D Group (N= 209)	3.4 (4.1)	2.9 (3.4)	-0.45(-0.84 to -0.07)		
Those without depression (PHQ-9 <5) at baseline					
Placebo Group (N= 151)	1.3 (1.3)	2.0 (2.6)	0.66 (0.27 to 1.04)	-0.24 (-0.79 to 0.30)	0.38
Vitamin D Group (N= 145)	1.4 (1.3)	1.9 (2.4)	0.41 (0.03 to 0.79)		
Those with at least mild depression (PHQ-9>=5) at baseline					
Placebo Group (N= 45)	8.9 (4.5)	6.7 (4.6)	-1.83 (-3.17 to -0.50)	-0.86 (-2.64 to 0.91)	0.34
Vitamin D Group (N= 56)	8.5 (4.4)	5.4 (4.3)	-2.70 (-3.86 to -1.53)		

Changes in outcomes are generated from mixed-effect models adjusted for age, sex and body mass index. Between-group differences were calculated by subtracting the vitamin D group values from the placebo group values.

The differences between the vitamin D supplementation and placebo groups became smaller but remained significant after further adjustment for WOMAC pain (β: -0.61, 95% CI: -1.17 to -0.06), and WOMAC function (β: -0.59, 95% CI: -1.15 to -0.02). The differences between the vitamin D status groups also diminished but remained significant after further adjustment for WOMAC pain (β: -0.64, 95% CI: -1.24 to -0.03) but became non-significant after further adjustment for WOMAC function (β: -0.59, 95% CI: -1.20 to 0.03). After further adjustment of change in season of blood sampling, the difference between vitamin D status groups maintained largely unchanged (β: -0.79, 95% CI: -1.47 to -0.10).

Figure 6.3 Change in PHQ-9 scores in the group that maintained vitamin D sufficiency between month 3 and 24 and the group which did not maintain vitamin D sufficiency between month 3 and 24



Vertical bars indicate 95% CIs for the mean scores.

P value was for the difference between the 2 groups in PHQ-9 score changes from baseline to month 2

Table 6.3 Effects of vitamin D status over 24 months on change in PHQ-9

	Baseline Mean (SD)	Month 24 Mean (SD)	Mean change Mean (95% CI)	Between-group difference change Mean (95% CI)	P-value
Whole sample					
Not maintaining sufficient vitamin D (N= 114)	2.4 (2.9)	2.9 (3.5)	0.36 (-0.20, 0.92)	-0.77 (-1.45, -0.08)	0.03
Maintaining sufficient vitamin D (N= 226)	3.3 (4.1)	2.8 (3.6)	-0.41 (-0.80, -0.02)		
Those without depression (PHQ-9 <5) at baseline					
Not maintaining sufficient vitamin D (N= 89)	1.4 (1.4)	2.0 (2.6)	0.60 (0.12, 1.08)	-0.15 (-0.75, 0.45)	0.62
Maintaining sufficient vitamin D (N= 161)	1.3 (1.3)	1.8 (2.4)	0.45 (0.09, 0.80)		
Those with at least mild (PHQ-9>=5) depression at baseline					
Not maintaining sufficient vitamin D (N= 18)	7.6 (2.9)	6.9 (3.9)	-0.76 (-2.70, 1.19)	-1.92 (4.14, 0.29)	0.09
Maintaining sufficient vitamin D (N= 61)	8.6 (4.5)	5.9 (4.6)	-2.68 (-3.74, -1.63)		

Changes in outcomes are generated from mixed-effect models adjusted for age, sex and body mass index, serum 25(OH)D level, PHQ-9 score, self-reported depression and usages of anti-depressant medication at baseline. Between-group differences were calculated by subtracting the maintaining sufficient vitamin D group values from the not maintaining sufficient group values.

6.3.2.2 Subgroup analysis by the presence of depression at baseline

In participants with depression (PHQ-9 score ≥5) at baseline, PHQ-9 scores improved significantly in the vitamin D supplementation group (N= 56), placebo group (N= 45) and the group that maintained sufficiency between month 3 and 24 (N= 61) (Table 2 and 3). There was no significant change in PHQ-9 scores in the group that did not maintain sufficiency (N= 18). While the differences between the groups were not statistically significant, PHQ-9 scores improved to a greater degree in the vitamin D supplementation group (β: -0.86, 95% CI: -2.64 to 0.91) and the group that maintained vitamin D sufficiency (β: -1.93, 95% CI: -4.14 to 0.28) between month 3 and 24 months compared to the placebo group and participants who did not maintain vitamin D deficiency, respectively. PHQ-9 scores worsened in all subgroups who did not suffer depression at baseline (Table 2 and 3). While there were no significant difference between the groups, the worsening was smaller in the vitamin D supplementation group and the group that maintained sufficient vitamin D between month 3 and 24.

In the participants with moderate to severe depression at baseline, the proportion who achieved MCID improvement (defined as >2.59/27) in PHQ-9 was 76.9% in the vitamin D supplementation group and 77.8% in the placebo group (P = 0.86).

6.4 Discussion

This current study, to the best of our knowledge, is the first to investigate the effect of vitamin D supplementation and maintaining vitamin D sufficiency on depressive symptoms over 24 months in patients with knee OA and vitamin D deficiency. In our sample, the prevalence of depression in knee OA patients was 25.4%. PHQ-9 scores improved in the vitamin D treatment group and the group which maintained vitamin D sufficiency between month 3 and 24, compared to the placebo group and the group which did not maintain sufficient vitamin D, respectively. Clinically significant improvement in depressive symptoms did not differ between treatment and placebo groups. Although the improvement was small, and the clinical importance was uncertain, vitamin D supplementation and maintaining vitamin D sufficiency could reduce depressive symptoms in patients with knee OA.

Despite the noticeable prevalence of depression in OA patients and the association between vitamin D deficiency and depression, no study has explored the effect of vitamin D supplementation on depression in OA patients. In addition, previous studies that assessed the effect of vitamin D supplementation on depression have not provided a consensus in the general population, obese or other diseased populations[162]. The inconsistencies among studies could be attributed to different study samples, different cut-offs for defining vitamin D deficiency, variations in the participants' baseline and follow-up vitamin D levels, and different methodologies used to evaluate depression[164]. Some researchers provided an explanation for their null findings stating that they did not measure 25(OH)D levels throughout the study to examine if supplementation actually changed serum 25(OH)D, which was described as "biological flaw" and should be considered as a limitation of the study design. Some studies failed to demonstrate whether participants were vitamin D deficient at baseline or whether they achieved sufficiency during the trial[162]. Further RCTs are needed to address this "biological flaw".

There was a pilot study which evaluated the effect of vitamin D supplementation on depression in patients with chronic widespread musculoskeletal pain (CWP) and vitamin D deficiency[271]. It demonstrated that 50,000IU/week oral vitamin D3 treatment for three months resulted in a prominent improvement in depression, which was assessed using the 21-item Beck Depression Inventory (BDI) scale. In the current study, we enrolled participants with vitamin D deficiency at baseline, and most participants achieved vitamin D sufficiency after 24 months of treatment with 50,000IU monthly vitamin D3. We found that the participants reaching vitamin D sufficiency at month 3 were significantly more in vitamin D supplementation group than in placebo group[150], and depressive symptoms were decreased more in vitamin D supplementation group from month 6. These suggest that vitamin D supplementation may have an effect on depressive symptoms when serum vitamin D levels reached optimal levels. Compared with placebo, vitamin D supplementation significantly reduced depressive symptoms (measured using the PHQ-9 score) in patients with knee OA in this study over 24 months. As we hypothesized, when the vitamin D deficiency were corrected, the vitamin D could have the neuroprotective effect on brain. Vitamin D supplementation with daily, weekly or monthly dosing frequencies can achieve equally well in vitamin D sufficiency[272]. Therefore, participants receiving a daily or weekly use of same cumulative dosage could result in a similar

outcome as receiving a monthly use. However, a monthly dosage can optimize participants' adherence in long-term vitamin D supplementation study as it is more convenient. One weakness of our study was that we included both depressed and non-depressed participants, though the prevalence of depression was high in our study population compared to the general population aged over 65[259].

In our RCT, 62% of participants in the placebo group reached a sufficient level of serum 25(OH)D at month 24 as reported previously, which was thought in part to result from seasonal variation, changes in lifestyle and dietary supplementation[235]. We hypothesized that the high proportion of patients achieving sufficient 25(OH)D levels in the placebo group would dilute the beneficial effects of vitamin D supplementation. Therefore, we performed a post-hoc analysis to describe whether maintaining sufficient serum vitamin D has a beneficial effect on depression. Indeed, our findings were consistent with our primary analysis, demonstrating maintaining sufficient serum vitamin D improved depressive symptoms. In addition, we found that the effects of vitamin D supplementation and maintaining serum vitamin D on depression remained largely unchanged after further adjustment for knee pain or function, suggesting that they were independent of improvements in knee symptoms.

There is limited information regarding a MCID for the PHQ-9 scale. *Lowe* defined a change of 2.59 as the MCID amongst individuals with major depression, dysthymia or both [205]; thus, we used this value as the cut-point to define a clinically important improvement in depression. In those with moderate to severe depression at baseline, we did not see any difference in the rate of those improving by treatment groups. However, it should be noted that the prevalence of mild and moderate to severe depression was 17.6% and 7.8%, respectively, in this current study, which was considerably lower than those in OA population from previous studies [273 274], indicating that a few participants were clinically depressed or had major depression. Due to a lack of MCID for those with milder depressive symptoms, it remains unclear whether the statistical significant improvement in depressive symptoms we reported in this study is clinically important. Notably, the effect size was doubled in those with any depression at baseline, suggesting the potential therapeutic effect of vitamin D would be most evident in knee OA patients suffering from depression.

This current study had some potential limitations. It was a secondary analysis of an RCT which was primarily designed to examine vitamin D supplementation on knee OA outcomes[150]. Nevertheless, this study is the first to examine the effect of vitamin D supplementation on depression in OA patients and the findings from this study are biologically plausible. While the effects were not significantly different in those with depression at baseline, most likely due to reduced sample size, the sizes were doubled in magnitude. It remains unclear whether the improvement in depressive syptoms we reported is clinically significant. Further RCTs are required to confirm our findings by selecting patients with both knee OA and depression. There may exsit unaticipanted selection bias, as baseline PHQ-9 score was higher in the group with maintainging vitamin D sufficiency. However, the associations remianed significant after adjustments for basline PHQ-9 score, suggesting the selection bias may not be a concern. In addition, 62% of patients in the placebo group reached sufficient vitamin D levels after 24 months[270], which might have been due to seasonal change, physical activity or other reasons. However, we used serum 25(OH)D levels to detect the association between variation in vitamin D status and depressive symptoms and obtained consistent findings. Furthermore, although the magnitude of the observed changes were samll, they are consistent in both parts with the RCT and obesevational study, suggesting the findings would be real.

In conclusion, vitamin D supplementation and maintaining sufficient vitamin D levels over 24 months may have beneficial effects on depressive symptoms in patients with knee OA.

Chapter 7 Risk factors for depression in knee OA patients

This manuscript has been submitted to "*British Journal of General Practice*" for review. The text of this chapter is the same as the submitted version. Thus, there are some repetitions of the methods.

7.1 Introduction

Osteoarthritis (OA) is a prevalent joint disease, characterised by whole joint structural changes, and is considered as "a serious disease"[3]. OA affects nearly 240 million people throughout the world, and its prevalence is projected to increase as the population ages and obesity rates increase[275]. Joint pain, stiffness and limited function are common joint symptoms of OA, resulting in reduced quality of life and disability that contribute to substantial financial burdens for individuals[24]. In addition, OA is associated with comorbidities, including cardiovascular disease, diabetes, hypertension, falls, fractures and depression[3].

Depression is a major global public health issue and is projected to be the second leading cause of disease burden by the year 2020[85 258]. A systematic review and meta-analysis recently reported that 19.9% of people with OA had depressive symptoms, with a relative risk of depression of 1.17 in those with OA compared to those without[86 87]. However, depression is often under-recognised and under-treated in older adults, particularly in patients with OA[259 276]. Furthermore, concomitant depression in OA patients contributes to increased difficulties in OA management and disease burden[85]. Therefore, the interaction between OA and depression should be taken seriously, and screening, prevention and treatment of depression in OA patients should be considered[277].

Concomitant depression with OA may be mediated by either biological or behavioural mechanisms with different aetiology and risk factors[85 278]. A better understanding of depression in OA patients is crucial for identifying modifiable risk factors and key areas for intervention[3]. Joint pain, decreased physical performance and increased risks for chronic comorbidities are typical characteristics of OA, which may be associated with depression[259 274 279]; however, the longitudinal relationship between clinical OA characteristics and depression in individuals with OA has been poorly studied. In addition, although the reciprocal relationship between depression severity and pain is established, whether current depression severity predicts changes in joint symptoms overtime has been rarely investigated in knee OA patients[280-282].

The aims of this study were, therefore, to describe demographic and clinical factors associated with the prevalence and incidence of depression and to explore the temporal relationship between depression and joint symptoms in patients with symptomatic knee OA.

7.2 Methods

7.2.1 Study design and participants

This study is a post-hoc analysis of the Vitamin D Effect on Osteoarthritis (VIDEO) study, which was a multicenter, randomized, double-blind, placebo-controlled trial to evaluate the effect of vitamin D supplementation in patients with symptomatic knee OA and vitamin D deficiency[175]. Participants were allocated to either the vitamin D or placebo arm at a ratio of 1:1 and received 50,000IU (1.25mg) vitamin D_3 (cholecalciferol) monthly for 24 months (treatment group) or identical inert placebo (placebo group)[175]. In this current study, both treatment and placebo groups were combined as a cohort.

Participants with symptomatic knee OA for at least 6 months, which was assessed according to the American College of Rheumatology (ACR) criteria[18], with knee pain of >20 mm on a 100-mm visual analog scale (VAS) and low levels of 25-hydroxyvitamin D [25(OH)D, between 12.5 and 60 nmol/L] were enrolled in Tasmania and Victoria, Australia[175]. Participants with the severe radiographic changes (grade 3 on the Altman and Gold atlas), severe knee pain on standing (>80 mm on a 100-mm VAS), other rheumatic diseases, contraindication to MRI, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, anticipated knee or hip surgery within the next 2 years and history of taking vitamin D within the previous 1 month were excluded from this study.

The study was approved by the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616). Informed written consent was obtained from all participants.

7.2.2 Measurements of depression severity

Depression severity was measured using the patient health questionnaire (PHQ-9) at baseline and 24 months. PHQ-9 is a valid and reliable instrument, which is widely used in diagnosing and assessing the severity of depression[205]. It is a nine-item depression questionnaire with a score range of 0 to 27, with each item being scored from 0 (not at all) to 3 (nearly every day). Using the mental health professional interview as the criterion standard, a cut-off point of ≥5

has a sensitivity of 81.5% and a specificity of 80.6% for mild depression and a cut-off point of ≥10 has a sensitivity of 54.3% and a specificity of 91.1% for moderate or severe depression[283].

7.2.3 Anthropometrics and social demographic characteristics

Height was measured to the nearest 0.1 cm (with shoes removed) using a stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Leicester, UK). Weight was measured to the nearest 0.1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307, Heine, New Hampshire, USA). Body mass index (BMI, in kg/m^2) was calculated[175].

Patients filled out a questionnaire which collected information on education history (grade 0: less than high school, grade 1: high school degree and grade 2: superior than high school degree), current smoking (yes or no) and concomitant medication usage.

7.2.4 Knee joint symptoms measurement

Knee joint symptoms were assessed at baseline and month 24 using the Western Ontario and McMaster Universities OA Index (WOMAC), which is widely used to evaluate the functional capacity in patients with OA with high performance in clinical trials [183]. The Index contains 24 questions, 5 related to pain, 2 to stiffness and 17 to physical function, with scores from 0 (none) to 100 (severe). The total WOMAC score (0-2400) is the sum of subscale scores including pain (0-500), stiffness (0-200) and physical function (0-1700).

7.2.5 Knee joint radiographic measurement

Radiographic OA was assessed at baseline using a standing semi-flexed anterior-posterior (AP) radiograph as per the Altman atlas[178]. Radiographs were assessed simultaneously by two observers using the Altman atlas to score osteophytes and joint space narrowing on a four-point scale (0 to 3) at medial tibial, lateral tibial, medial femoral and lateral femoral sites. The presence of radiographic OA was defined as any score of ≥ 1 for JSN or osteophytes.

7.2.6 Physical activity

Physical activity was assessed at baseline and month 24 as steps/day determined by pedometer (SW200 Digi-Walker, Yamax Corporation, Tokyo, Japan)[175]. Briefly, participants were instructed to wear a pedometer for 7 consecutive days from the time they woke up until they went to bed and to record the number of steps each day and the duration and type of physical activity for any activities in which the pedometer could not be worn (for example, swimming). Mean steps/day were calculated as the average of the days worn at both time points[202].

7.2.7 Multi-sites pain

The location of sites at which the participants experienced pain was self-reported at baseline. Participants were asked whether they had pain (yes/no) in the following sites: neck, lower back, hands, shoulders and others. The total number of painful sites (range 0 to 5) was categorized into 3 groups (no pain, one painful site, two or more painful sites) according to the number of painful site groups.

7.2.8 Self-reported medical conditions

Participants were asked whether they have been told by a doctor or a nurse that they had (yes/no) any of the following conditions: depression, angina, high blood pressure, heart attack, stroke, high cholesterol, diabetes, osteoporosis, asthma, bronchitis and emphysema, and whether they had these conditions currently. The total number of current comorbidities, except for depression, was categorized into three groups (no comorbidity, one comorbidity and two or more comorbidities).

7.2.9 Data analyses

Baseline characteristics are described as mean ± standard deviation (SD) or numbers of participants (percentage). Univariable and multivariable log-binomial regressions were used to explore risk factors associated with the prevalence of depression at baseline and incidence of depression over time. If the log-binomial model failed to converge, it was estimated by using a Poisson distribution and robust standard errors. Multivariable models for prevalent depression were adjusted for age, sex, BMI and baseline vitamin D level. Multivariable models for incident depression were adjusted for age, sex, BMI, baseline 25(OH)D level and treatment

arms (vitamin D treatment versus placebo). Univariable and multivariable linear regressions were used to examine the temporal relationship between baseline depressive symptoms and change in knee symptoms over 24 months before and after adjustment for age, sex, BMI, baseline 25(OH)D level, treatment arms and baseline WOMAC score. All tests were two-sided and a *P* value of <0.05 was considered statistically significant. Stata version 12.0 was used to perform statistical analyses.

7.3 Results

7.3.1 Baseline characteristics of participants

Table 7.1 presents baseline characteristics of the study participants. The mean age of 413 participants was 63.2 ± 7.0 years and mean BMI was 29.6 ± 5.0. Of them, 208 (50.4%) participants were female and 209 (50.6%) were allocated to the treatment group. At baseline, 296 (74.6%) participants were identified as not suffering from depression, 70 (17.6%) were identified as suffering from mild depression, and 31 (7.8%) were identified as suffering from moderate to severe depression. The prevalence of any depression in this study was 25.4% (according to the PHQ-9), 5.8% of participants had self-reported depression and 4.3% used anti-depressant medications.

Table 7.1 Baseline characteristics of participants (N= 413)

	Mean/ Numbers	SD/ Percentage
Age (years)	63.2	7.0
Female sex (n, %)	208	50.4
Body mass index (kg/m^2)	29.6	5.0
Serum 25-(OH)D levels (nmol/L)	43.8	12.2
PHQ score		
0-4	296	74.6

5-9	70	17.6
≥ 10	31	7.8
Anti-depressant medication use (n, %)	17	4.3
Physical activity, (1000 step/day)	7.4	3.4
Education		
School only (n, %)	51	16.3
High school (n, %)	101	32.3
University or higher (n, %)	161	51.4
Radiographic osteoarthritis (n, %)	339	96.0
WOMAC score		
Pain (0-500)	136.3	86.1
Function (0-1700)	478.0	305.8
Stiffness (0-200)	57.4	16.6
Multi-site joint pain, (n, %)		
No pain	252	61.0
One site	136	32.9
More than one site	25	6.1
Comorbidity, (n, %)		
No comorbidity	170	41.2
One comorbidity	229	55.5
More than one comorbidity	14	3.4

PHQ-9, patient health questionnaire depression scale.

7.3.2 Factors associated with the prevalence of depression

As shown in Table 7.2, being female, and having a higher BMI, greater scores of WOMAC pain (Figure 7.1 A), WOMAC function (Figure 7.1 B) and WOMAC stiffness, a lower education level, and one more comorbidity were significantly associated with a higher prevalence of depression in univariable analyses. After adjustment for age, sex, BMI and baseline 25-(OH)D level, the associations persisted except for female sex, which was no longer statistically significant. Having two or more sites pain appeared to be significantly associated with higher prevalence of depression in multivariable analyses. Age and radiographic OA were not significantly associated with the prevalence of depression in either univariable or multivariable analysis. Results were similar, when self-reported depression was used as the outcome or if the analyses were adjusted for anti-depressant medication use (data not shown).

Table 7.2 Factors associated with the prevalence of mild to severe depression at baseline

	Univariable PR (95% CI)	Multivariable* PR (95% CI)
Age (years)	0.99 (0.98, 1.01)	0.96 (0.94, 0.99)
Female sex (n, %)	1.55 (1.10, 2.18)	1.38 (0.98, 1.96)
BMI (kg/m^2)	1.06 (1.03, 1.09)	1.05 (1.02, 1.08)
Physical activity, (1000 steps/day)	0.96 (0.92, 1.01)	0.96 (0.90, 1.03)
Education		
School only (n, %)	*Reference*	*Reference*
High school (n, %)	0.63 (0.40, 1.00)	0.61 (0.40, 0.95)
University or higher (n, %)	0.47 (0.30, 0.73)	0.50 (0.34, 0.74)
Radiographic osteoarthritis (n, %)	0.98 (0.94, 1.02)	0.98 (0.94, 1.02)
WOMAC score/ 10 unit		
Pain (0-50)	1.04 (1.03, 1.07)	1.05 (1.03, 1.07)

Function (0-170)	1.02 (1.01, 1.02)	1.02 (1.01, 1.02)
Stiffness (0-20)	1.07 (1.04, 1.11)	1.05 (1.02, 1.09)
Multi-site joint pain, (n, %)		
No pain	*Reference*	*Reference*
One site	1.21 (0.85, 1.74)	1.06 (0.75, 1.51)
More than one site	1.64 (0.93, 2.88)	1.73 (1.01, 2.98)
Comorbidity, (n, %)		
No comorbidity	*Reference*	*Reference*
One comorbidity	1.08 (0.75, 1.55)	1.14 (0.80, 1.65)
More than one comorbidity	2.13 (1.18, 3.86)	1.98 (1.03, 3.80)

Mild to severe depression was defined as PHQ-9 scores ≥ 5.

*All multivariable analyses were adjusted for age, sex, BMI and baseline 25-(OH)D level, except for age (adjusted for sex, BMI and baseline 25-(OH)D level), sex (adjusted for age, BMI and baseline 25-(OH)D level) and BMI (adjusted for age, sex and baseline 25-(OH)D level).

7.3.3 Factors associated with incidence of depression

340 participants completed the follow-up. 28 out of 249 participants (11.2%) without depression at baseline reported having incident mild to severe depression at 24 months. Table 7.3 shows the factors associated with incident depression over 24 months. Being female and having a higher WOMAC pain (Figure 7.1 C) and WOMAC function (Figure 7.1 D) score were significantly associated with greater incident depression over 24 months in the univariable and multivariable analyses. Having two or more painful sites was significantly associated with greater incident depression over 24 months in the multivariable analyses. In a sensitivity analysis further adjusting for anti-depressant medication, the results were largely unchanged (data not shown). In contrast, there were no significant associations between age, BMI,

physical activity, education, radiographic OA, WOMAC stiffness, comorbidity and incident depression.

Table 7.3 Factors associated with the incidence of mild to severe depression at 24 months amongst participants without depression at baseline

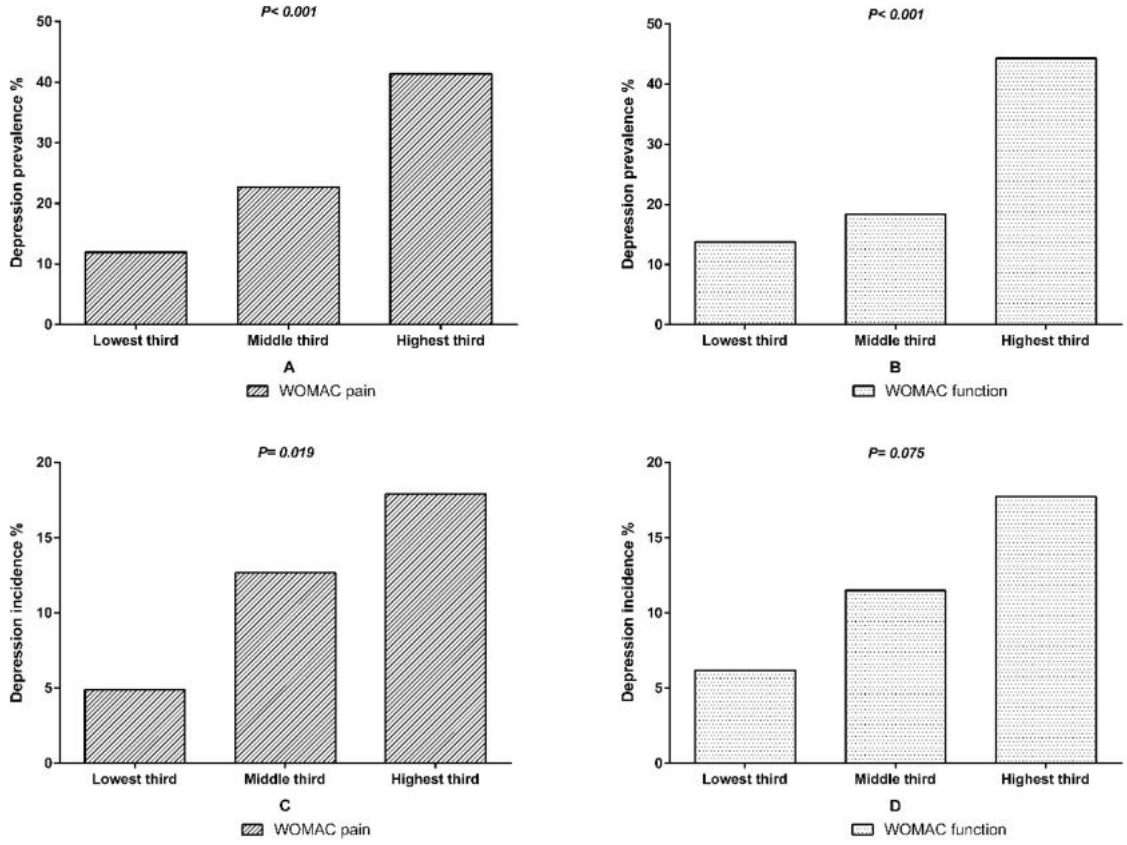
	Univariable	Multivariable*
	RR (95% CI)	RR (95% CI)
Age (years)	0.99 (0.94, 1.04)	1.00 (0.96, 1.05)
Female sex (n, %)	2.23 (1.06, 4.66)	2.33 (1.09, 4.95)
Body mass index (kg/m^2)	0.98 (0.91, 1.06)	0.98 (0.91, 1.06)
Physical activity, (steps/day)	1.01 (0.90, 1.13)	0.99 (0.87, 1.12)
Education		
School only (n, %)	*Reference*	*Reference*
High school (n, %)	0.86 (0.24, 3.10)	0.81 (0.22, 2.94)
University or higher (n, %)	0.89 (0.28, 2.82)	0.89 (0.28, 2.84)
Radiographic osteoarthritis (n, %)	0.92 (0.84, 1.01)	0.93 (0.85, 1.02)
WOMAC score/ 10 unit		
Pain (0-50)	1.04 (1.00, 1.08)	1.04 (1.00, 1.08)
Function (0-170)	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)
Stiffness (0-20)	1.01 (0.93, 1.11)	1.01 (0.93, 1.10)
Multi-site joint pain, (n, %)		
No pain	*Reference*	*Reference*
One site	1.30 (0.61, 2.75)	1.13 (0.53, 2.42)
More than one site	2.17 (0.69, 6.80)	3.63 (1.04, 12.7)
Comorbidity, (n, %)		

No comorbidity	*Reference*	*Reference*
One comorbidity	0.93 (0.45, 1.91)	1.01 (0.48, 2.14)
More than one comorbidity	1.12 (0.16, 7.68)	0.97 (0.14, 6.87)

Mild to severe depression was defined as PHQ-9 scores ≥ 5.

*All multivariable analyses were adjusted for age, sex, BMI and baseline 25-(OH)D level, except for age (adjusted for sex, BMI and baseline 25-(OH)D level), sex (adjusted for age, BMI and baseline 25-(OH)D level) and BMI (adjusted for age, sex and baseline 25-(OH)D level).

Figure 7.1 Associations of baseline WOMAC pain (0-500) and WOMAC function (0-1700) tertiles with the prevalence (A, B) and incidence (C, D) of depression in patients with knee OA



7.3.4 Temporal relationship between baseline depression and changes in joint symptoms overtime

Table 7.4 describes the longitudinal association of baseline depression with changes in knee joint symptoms over 24 months. Although participants with mild to severe depression had greater decreases in WOMAC symptoms compared to participants without depression at baseline in the univariable analyses, the significant associations disappeared in the multivariable analyses. In addition, the results were consistent when either self-reported depression at baseline was used as the exposure or if the analyses were adjusted for anti-depressant medication use (data not shown).

Table 7.4 The association between depression severity at baseline and change in joint symptom over 24 months

	Univariable β (95% CI)	Multivariable β (95% CI)
WOMAC Pain (0-500) †		
No depression	*Reference*	*Reference*
Mild depression	-35.8 (-63.7, -7.9)	-12.4 (-36.7, 11.8)
Moderate to severe depression	-100.3 (-143.1, -57.4)	-32.0 (-72.2, 8.2)
WOMAC Function (0-1700) ‡		
No depression	*Reference*	*Reference*
Mild depression	-139.9 (-222.2, -57.7)	-67.8 (-145.4, 9.7)
Moderate to severe depression	-225.4 (-357.5, -93.2)	-90.3 (-220.3, 39.7)
WOMAC Stiffness (0-200) §		
No depression	*Reference*	*Reference*
Mild depression	-13.2 (-26.0, -0.5)	-5.2 (-16.5, 6.1)
Moderate to severe depression	-29.4 (-49.0, -9.8)	-11.8 (-30.0, 6.5)

† Multivariable analysis was adjusted for age, sex, BMI, baseline 25-(OH)D level, treatment arm and baseline WOMAC pain score.

‡ Multivariable analysis was adjusted for age, sex, BMI, baseline 25-(OH)D level, treatment arm and baseline WOMAC function score.

§ Multivariable analysis was adjusted for age, sex, BMI, baseline 25-(OH)D level, treatment arm and baseline WOMAC stiffness score.

7.4 Discussion

This study investigated the temporal relationships between demographic and OA clinical factors, joint symptoms and depression in patients with symptomatic knee OA. Depression was common in this population with a prevalence of 25.4% and incidence of 11.2% over 24 months. Common OA risk factors such as higher BMI, lower education level and having two or more comorbidities were associated with prevalent depression and being female was associated with incident depression in knee OA patients. Higher levels of knee pain and physical dysfunction and having multi-site pain were associated with increased risks of both prevalent and incident depression. In contrast, baseline depression severity did not predict changes in OA symptoms. These findings suggest that treatment of depression in OA may not necessarily lead to improvements in knee symptoms in knee OA patients.

Numerous studies have explored the demographic factors associated with depression in elderly populations. Female sex, lower education levels and the biological risks including endocrine and inflammatory factors are potential risk factors for depression in the elderly[284]. However, only a few studies have been conducted in individuals with OA, and most did not assess longitudinal relationships. A cross-sectional study reported that fewer social contacts, increased BMI, perceived pain and limited physical activity were associated with depression severity in 1021 patients with knee OA[274]. In our current study, we reported similar results and reported that, cross-sectionally, higher BMI, lower education level and having two or more comorbidities were associated with prevalent depression.

Obesity, female sex and a lower education level are known as risk factors for both OA and depression. Therefore, the high prevalence of depression in OA individuals may be in part

attributed to shared risk factors. Taking higher BMI for example, higher BMI, indicating overweight or obesity, is a well-known risk factor of OA and also can increase vulnerability to depression directly and indirectly through complex mechanisms[285 286]. Obesity causes a high prevalence of OA, which may be related to combined effects from biomechanical, inflammatory and metabolic factors. In addition, obesity can induce poor self-image, low self-esteem and social isolation, which are well-known contributors to depression. It also can activate inflammatory pathways, involving hypothalamic-pituitary-adrenal axis dysregulation, which are associated with increased risk of depression through biological and psychological pathways[285]. Obesity and lower levels of education are modifiable factors; therefore, management of obesity and improvement of education level may prevent depression and should have beneficial effects in patients with knee OA. Additionally, female sex predicted incident depression over 24 months in knee OA patients. Hence, it is important for clinicians to screen for, and try to prevent and treat depression, especially in female patients with knee OA.

Joint pain and dysfunction, multisite pain and comorbidities are clinical characteristics of OA. Multisite pain, joint pain and joint function limitation are common in musculoskeletal conditions, and have been linked to depression in previous studies[287]. Individuals who experienced chronic pain and physical activity limitation are at an increased risk of depression[288 289]. In OA patients, joint pain severity and dysfunction were associated with depression severity cross-sectionally and longitudinally[290-295]. Knee OA patients with greater pain associated with higher risk of depression at baseline and slow gait speed may represent an important risk factor for worsening depressive symptoms over time[293 295]. In our study, although we used a different method to assess depression severity, we found similar results. Individuals who experienced multi-site pain, more severe knee pain and joint dysfunction, and had more than one comorbidity were at a higher risk of prevalent depression cross-sectionally in knee OA patients. Over 24 months, multi-site pain, knee pain and dysfunction were associated with increased incidence of depression over 24 months, suggesting a potential causal relationship. These supported the notion that management of pain, joint dysfunction and other comorbidities may help to improve depression in knee OA patients[281 296].

Depression severity is dynamic, changing over time, and prior depressive illness modifies the experience of currently depressed mood[297]. The reciprocal relationship between depression severity and pain severity has been well established, but whether current depression severity has causal effects on severity of joint symptoms overtime has been rarely investigated[280 282]. Kurt et al have reported that change in pain severity over 3 months predicted subsequent depression severity, and vice versa, change in depression severity over 3 months predicted subsequent pain severity over 12 months in patients with persistent back, hip and knee pain[280]. In knee OA patient, we only found higher levels of knee joint symptoms and having multi-site pain at baseline were associated with increased risks of both prevalent and incident depression, while baseline depression severity did not predict knee joint symptomatic progression over two years. There was one study have explored the causal cumulative effect between depressive symptoms and knee pain among patients with knee radiographic OA. Rathbun reported the causal effect of pain severity significantly increases with the persistence of depressed mood, but depressive symptoms on OA knee pain does not change over time, which was smiliar with this current study[282]. Our findings suggest the potentially cause-effect relationship is from knee joint pain and physical dysfunction to depression, rather than from depression to knee symptoms, in patients with knee OA.

This study has some potential limitations. It was a post-hoc analysis of an RCT which was primarily designed to examine vitamin D supplementation on knee OA outcomes. Nevertheless, the findings from this study were plausible as we found that vitamin D supplementation reduced visual analog scale knee pain, improved physical function and decreased depressive symptoms[150 298]. The effects on depressive symptoms may be mediated through the effects on knee pain and physical function. In addition, 18% participants did not complete the 24 months follow-up, and loss of follow-up bias may be present; however, the retention rate in this trial was high, and there were no significant differences in the baseline characteristics between those who completed and who did not complete the trial. This suggests a minimal loss of follow-up bias in our study. Furthermore, we defined depression using the patient health questionnaire, which was developed for depression diagnostic, severity measures and assessment of depression outcome changes over time. It may lead to misclassification of depression; however, when we used the self-reported depression as the outcome or exposure and anti-depressant medication as a covariate and the results were largely unchanged.

In summary, knee OA risk factors and joint symptoms, along with co-existing multi-site pain are associated with the prevalence and development of depression. This provide empirical evidence that managing common OA risk factors and joint symptoms could be important for prevention and treatment depression in patients with knee OA.

Chapter 8 Effects of vitamin D on foot pain

This manuscript has been submitted to "*Seminars in Arthritis and Rheumatism*" for review. The text of this chapter is the same as the submitted version. Thus, there are some repetitions of the methods.

8.1 Introduction

Osteoarthritis (OA) is a highly prevalent chronic joint disease worldwide characterized by joint pain and deformity. The knee is the most common affected joint and is often associated with dsiabilty[22]. In those over 60 years old, the global prevalence of symptomatic knee OA is approximately 10% in men and 13% in women[1], and the prevalence of knee OA has estimately doubled since the mid-20th century[299], and the financial burden is estimated as high as 1.0%-2.5% of the GDP in Western countries[300]. Although there have been effective agents that can relieve pain and improve function, there are no treatments showing significant disease-modifying effects on OA disease progression[301 302]. More important, in older adults, comorbidity for OA was prevalent, with musculoskeletal as well as non-musculoskeletal conditions[83].

Foot pain, a common musculoskeletal pain, affects nearly one in five older people in the community and has a detrimental impact on health-related quality of life[91 92 96 208 303]. Foot pain often coexists with knee pain, further impairing physical activity, quality of life and increased levels of depression in knee OA patients than in the general population[93 96]. Moreover, foot pain is a prevalent commorbidty in knee OA patients. In the OA initiative cohort study, 25% knee OA patient reported occurrent foot pain[93]. A survey of 8990 older people has demonstrated most people with knee pain had multiple joint site pain, and the severity of knee pain and related disability were worse in the presence of pain elsewhere[304]. Given that patients with knee OA are more likely to have more severe foot pain and foot pain often lead to even worse physical activity and lower quality of life in knee OA patients, management of foot pain in OA patients is of priority.

Previous epidemiological studies reported that vitamin D deficiency was associated with chronic musculoskeletal pain and depression, but underlying mechanisms were complex and unclear[159 167 305]. Studies exploring the effect of vitamin D supplementation on non-specific chronic pain in the adult population and those with rheumatoid arthritis and osteoporosis have had inconsistent findings[167 306]. To date, there have been no studies exploring the effect of vitamin D supplementation on foot pain in knee OA patients. Therefore, our study aims to explore whether vitamin D supplementation or maintaining sufficient vitamin D level reduces foot pain in patients with symptomatic knee OA, initially with lower vitamin D status.

8.2 Methods

8.2.1 Participants and trial design

A post hoc study was conducted from a randomized double-blind placebo-controlled trial named the Vitamin D Effect on Osteoarthritis (VIDEO) study, in which the primary outcomes were tibial cartilage volume and knee pain among patients with symptomatic knee OA[307]. Patients who suffered from symptomatic knee OA at least for 6 months, had knee pain of 20-80 mm on a 100-mm visual analog scale (VAS) and serum 25-hydroxyvitamin D levels between 12.5 nmol/L to 60 nmol/L, and aged 50 to 79 years were included. Patients with the Altman and Gold atlas grade 3 radiographic changes, severe knee pain on standing (>80 mm on a 100-mm VAS), other rheumatic diseases such as rheumatoid arthritis, psoriatic arthritis and lupus, contraindication to MRI, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, anticipated knee or hip surgery within the next 2 years and history of taking vitamin D within the previous 1 month were excluded from this study. After signing the written consent, patients were randomly allocated to 24 months' vitamin D or placebo treatment at a ratio of 1:1. A monthly capsule containing 50000IU (1.25mg) vitamin D3 (cholecalciferol) or placebo was given to patients and assessments were conducted at baseline and at month 3, 6, 12 and 24.

8.2.2 Measurements

8.2.2.1 Manchester Foot Pain and Disability Index Questionnaires

Manchester Foot Pain and Disability Index (MFPDI) questionnaire was used to measure foot pain of patients at month 0, 3, 6, 12 and 24. MFPDI was developed to measure foot pain and disability in the elderly[308]. Each item was scored either 1 (none of the time), 2 (on some days) or 3 (on most days/every day). The total score was calculated by summing the scores of 17 items and higher score indicated greater disability. Four subscales were calculated including functional limitation (items 2-8), pain intensity (items 10-11, 14-17), concern about appearance (items 12-13) and activity restriction (items 1 and 9)[208]. It has been proven to be a useful and valid instrument of assessing foot pain in the older population and was used in both observational studies and randomized controlled trials[309]. Disabling foot pain was defined when at least one of the 10 functional limitation items (items 1-9,11) being documented as on 'most/every day(s)' in the last month[309].

8.2.2.2 Knee structures measurements

Radiographic OA was assessed at baseline by a standing semiflexed anterior-posterior radiograph as per the Altman atlas[177] according to the protocol using the Osteoarthritis Research Society International (OARSI) atlas to score osteophytes and joint space narrowing. MRI scans with a commercial transmit-receive extremity coil at baseline and two years of the study knee were obtained according to a standardized protocol. T2-weighted/proton density-weighted fast spin echo sequences were used to assess cartilage defects and bone marrow lesions (BMLs) and detail are described before in the protocol[310].

8.2.2.3 Knee symptoms measurements

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[183] scale was used to detect knee symptoms at month 0, 3, 6, 12 and 24. The sum of pain (0-500), stiffness (0-200) and physical function (0-1700) subscales is calculated as the total WOMAC score (0-2400).

8.2.2.4 Serum 25(OH)D measurement and definition of vitamin D status

Serum 25-hydroxyvitamin D levels were assayed using direct competitive chemiluminescent immunoassays at screening, month 3 and month 24. Patients whose serum 25-hydroxyvitamin D level greater than 50 nmol/L at both month 3 and 24 were classified into maintaining sufficient vitamin D group and whose serum 25-hydroxyvitamin D level less than 50 nmol/L at either month 3 and 24 were classified into not maintaining sufficient vitamin D group.

8.2.2.5 Other measurements

Body height and weight were measured at baseline and body mass index (BMI, in kg/m2) was calculated. Height was measured to the nearest 0.1 cm (with shoes removed) using a stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Leicester, UK) and weight was measured to the nearest 0.1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307, Heine, New Hampshire, USA).

8.2.3 Statistical analysis

Baseline characteristics between patients with and without disabling foot pain were compared using independent t or χ^2 tests. A repeated-measure mixed effect model with terms for

treatment, time, treatment by time and adjustment for age, sex, BMI was used to analyse the change in MFPDI scores over 24 months between groups including vitamin D vs. placebo group and maintaining sufficient vitamin D vs. not maintaining sufficient vitamin D group. Multilevel mixed-effect models were used to deal with missing data caused by loss to follow-up and nonresponses. Subgroup analysis exploring effects of vitamin D supplementation and maintaining sufficient vitamin D level on foot pain relief in patients with disabling foot pain at baseline was performed. All tests were two-sided and P value <0.05 was considered statistically significant. Stata version 12.0 was used to perform statistical analyses.

8.3 Results

8.3.1 Baseline characteristics

A total of 413 patients were included and randomized to receive either vitamin D (n=209) or placebo (n=204) treatment. After 24 months, 340 patients completed the trial and no significant differences in baseline characteristics were found between patients who completed the study and who did not. The average age of patients was 63.2 years with a mean BMI of 29.6, and 49.7% of them were female. The mean MFPDI score was 22.8±7.3. For those reported foot pain (n=214, 51.8%) at baseline, 74 (34.6%) reported pain in the toes, 49 (22.9%) reported pain in the ball of foot, 48 (22.4%) reported pain in the arch, 43 (20%) reported in whole feet and 37 (17.3%) reported pain in heel. 23.7% (n=98) of patients were found to have disabling foot pain according to the MFPDI case definition. There were also no significant differences in baseline characteristics, MFPDI scores, prevalence of disabling foot pain, knee symptoms and knee structure measurements between vitamin D group and placebo group (Table 1). At the same time, the baseline vitamin D level was higher in the maintaining sufficient vitamin D group compared with not maintaining sufficient vitamin D group (45.2 vs 41.5, P=0.01) while no significant differences were found between groups in other characteristics.

Table 8.1 Baseline characteristics of participants between maintaining sufficient vitamin D group and insufficient vitamin D group

	Vitamin D group N= 209	Placebo group N= 204	P value
Age, mean (SD), years	63.6 (6.9)	62.6 (7.2)	0.32
Female, No. (%)	106 (50.7)	102 (50)	0.92
Body mass index, mean (SD),kg/m^2	29.6 (5.4)	29.6 (4.6)	0.88
Serum 25-(OH) D levels, mean (SD), nmol/L	43.7 (11.8)	43.8 (12.7)	0.95
MFPDI (0-34), mean (SD)	21.6 (6.8)	22.7 (7.5)	0.27
Disabling foot pain, No. (%)	101 (47.4)	112 (52.6)	0.30
AQOL (0-1), mean (SD)	0.74 (0.17)	0.75 (0.18)	0.69
*Depression, No. (%)	56 (27.9)	45 (23.0)	0.26
WOMAC score			
Pain (0-500)**,** mean (SD)	137.9 (88.8)	134.7 (83.4)	0.71
Function (0-1700)**,** mean (SD)	487.9 (318.1)	467.6 (292.8)	0.50
Stiffness (0-200)**,** mean (SD)	61.5 (41.5)	61.7 (40.1)	0.95
Knee structures			
Total radiographic OA (0-18)	8.3 (5.6)	8.3 (4.9)	0.93
Total cartilage defects (0-24)	14.8 (4.1)	14.4 (3.9)	0.29
Total bone marrow lesions (0-45)	3.2 (3.2)	3.6 (3.2)	0.17

MFPDI, Manchester foot pain and disability index; AQoL, assessment of quality of life.

Depression was defined as PHQ-9 score of ≥5.

Two-tailed Student's t-tests were used for differences between means.

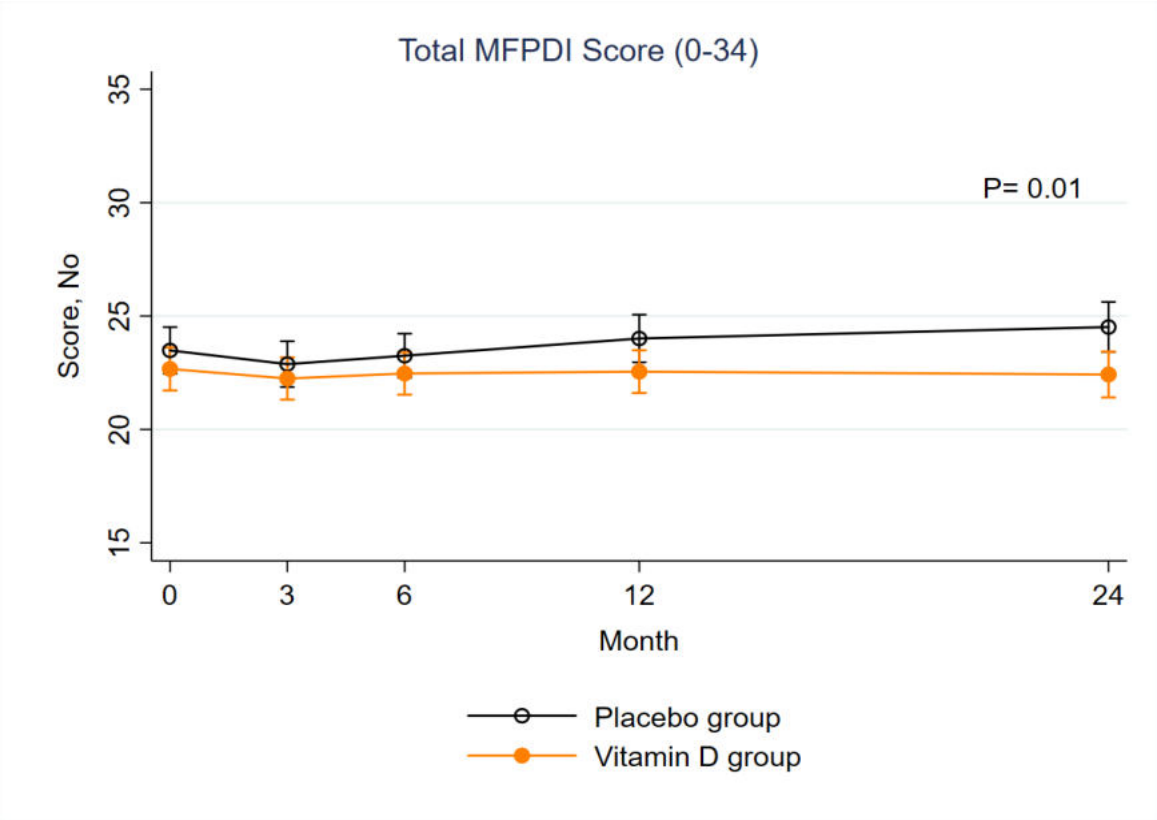
$\chi 2$ tests were used for proportions (percentages) and Wilcoxon rank-sum tests were used for differences between medians.

8.3.2 Vitamin D supplementation and change in MFPDI scores

Over 24 months, MFPDI scores remained largely unchanged in the vitamin D group (β: -0.03, 95% CI: -0.80 to 0.74) while worsened in the placebo group (β: 1.30, 95% CI: 0.51 to 2.09) (Figure 8.1), and there were significant differences in change of MFPDI scores between groups in the mixed-effect model after including all time points adjusted for age, sex and BMI (-0.03 in vitamin D group vs. 1.30 in placebo group; between-group difference, β: -1.32, 95% CI: -2.43 to -0.22, P= 0.013) (Table 8.2 and Figure 8.1).

In subgroup analyses, for patients with disabling foot pain at baseline, although those who received vitamin D treatment (β: -4.86, 95% CI: -6.79 to -2.93) had greater reduction in MFPDI scores compared with placebo group (β: -2.30, 95% CI: -5.33 to -0.31) after 24 months, no significant difference between groups was found (β: -2.56, 95% CI: 5.33 to 0.21, P= 0.07) (Table 8.2).

Figure 8.1 Change in MFPDI scores in the vitamin D supplementation group and the placebo group



Vertical bars indicate 95% CIs for the mean scores.

P value was for the difference between the 2 groups in MFPDI score changes from baseline to month 24.

Table 8.2 Effect of vitamin D supplementation on change in MFDPI over two years

	Mean change Mean (95% CI)	Between-group difference change Mean (95% CI)	P value
Whole sample			
Placebo Group (N= 204)	1.30 (0.51, 2.09)	-1.32 (-2.43, -0.22)	0.013
Vitamin D Group (N= 209)	-0.03 (-0.80, 0.74)		
Those without disabling foot pain at baseline			
Placebo Group (N= 153)	2.46 (1.66, 3.25)	-1.09 (-2.20, 0.02)	0.05
Vitamin D Group (N=162)	1.36 (0.59, 2.14)		
Those with disabling foot pain at baseline			
Placebo Group (N=51)	-2.30 (-5.33, -0.31)	-2.56 (-5.33, 0.21)	0.07
Vitamin D Group (N=47)	-4.86 (-6.79, -2.93)		

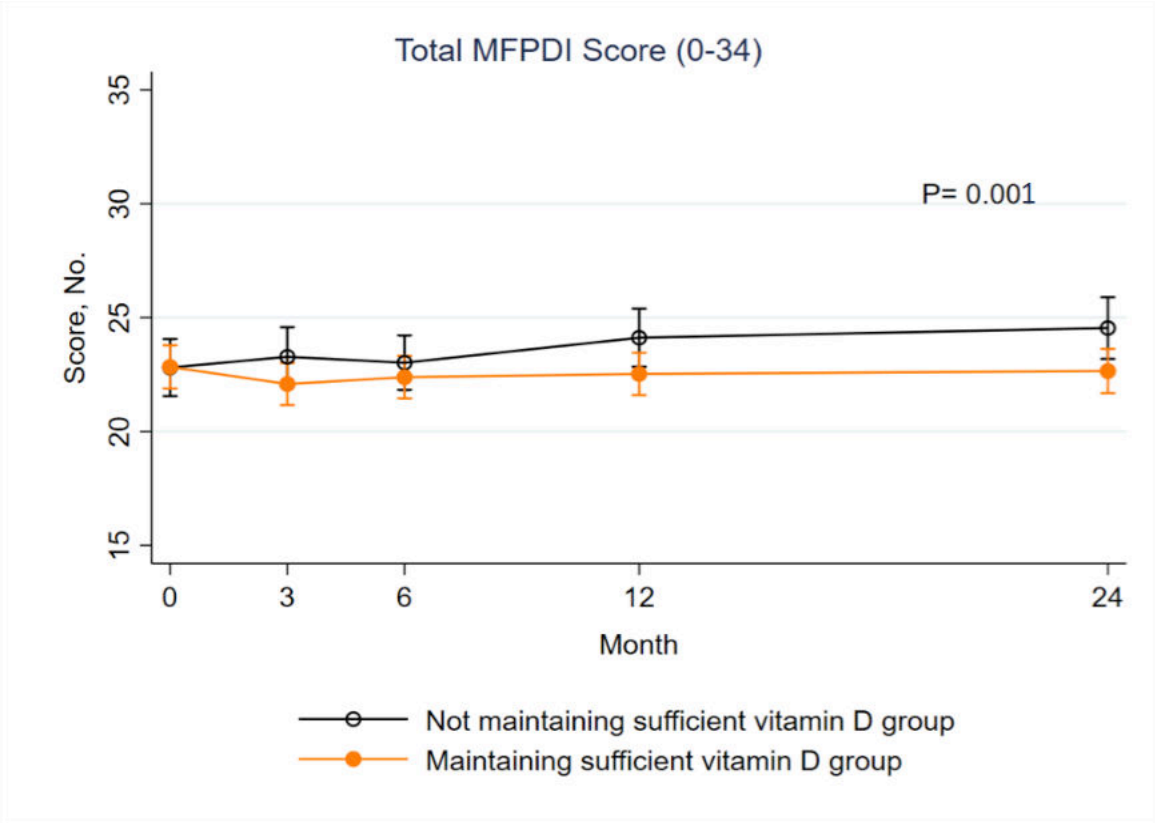
Changes in outcomes are generated from mixed-effect models adjusted for age, sex and body mass index. Between-group differences were calculated with vitamin D group values minus placebo group values.

8.3.3 Maintaining sufficient vitamin D levels and change in MFPDI scores

Post-hoc analyses comparing patients who maintained sufficient vitamin D with who did not maintain vitamin D sufficiency showed that MFPDI score decreased in those maintaining sufficient vitamin D group (β: -0.09, 95%CI: -0.79 to 0.61) while increased in those not maintaining sufficient vitamin D group (β: 2.19, 95%CI: 1.21 to 3.81) over 2 years (between-group difference, β: -2.29, 95%CI: -3.49 to -1.08, P=0.001) after adjusted for age, sex, BMI, serum 25(OH)D level and baseline MFPDI score (Table 8.3 and Figure 8.2). The results maintained largely unchanged after further adjustment for the change in season of blood sampling (β: -2.30, 95% CI: -3.52 to -1.10).

In subgroup analyses, for those with disabling foot pain at baseline, there was a greater decrease in MFPDI score in those maintaining sufficient vitamin D group (β: -4.63, 95% CI: -6.35 to -2.92) compared to those not maintaining sufficient vitamin D group (β: -0.14, 95% CI: -2.75 to 2.48) and between-group difference (β: -4.49, 95% CI: -7.62 to -1.37, P= 0.005) was significant, as shown in Table 8.3. There were similar findings in patients without disabling foot pain at baseline (Table 8.3).

Figure 8.2 Change in MFPDI scores in the group that maintained vitamin D sufficiency between month 3 and 24 and the group which did not maintain vitamin D sufficiency between month 3 and 24



Vertical bars indicate 95% CIs for the mean scores.

P value was for the difference between the 2 groups in MFPDI score changes from baseline to month 24.

Table 8.3 Effect of vitamin D status on change in MFDPI over 2 years

	Mean change Mean (95% CI)	Between-group difference change Mean (95% CI)	P value
Whole sample			
Insufficient vitamin D (N= 114)	2.19 (1.21 to 3.18)	-2.29 (-3.49 to -1.08)	0.001
Maintaining sufficient vitamin D (N= 226)	-0.09 (-0.79 to 0.61)		
Those without disabling foot pain at baseline			
Insufficient vitamin D (N= 91)	2.76 (1.77, 3.74)	-1.55 (-2.76, -0.33)	0.01
Maintaining sufficient vitamin D (N= 174)	1.21 (0.50, 1.93)		
Those with disabling foot pain at baseline			
Insufficient vitamin D (N= 23)	-0.14 (-2.75, 2.48)	-4.49 (-7.62, -1.37)	0.005
Maintaining sufficient vitamin D (N= 52)	-4.63 (-6.35, -2.92)		

Changes in outcomes are generated from mixed-effect models adjusted for age, sex, body mass index, serum 25(OH)D level and baseline MFPDI score. Between-group differences were calculated with maintaining sufficient vitamin D group minus insufficient group.

8.4 Discussion

To the best of our knowledge, this current study is the first to investigate the effects of supplementing vitamin D and maintaining sufficient vitamin D level on foot pain in symptomatic knee OA patients. In this sample, 51.8% of participants with knee OA and vitamin D deficiency reported foot pain. MFPDI scores decreased more in the vitamin D treatment group and maintaining sufficient vitamin D group, compared to the placebo group and not maintain sufficient group, respectively, over 24 months. Our results suggest that foot pain is common and vitamin D supplementation and maintaining sufficient vitamin D levels over 24 months may have beneficial effects on foot pain in patients with knee OA.

Foot pain is a common condition in patients with OA. A recent cross-sectional study using data from the Osteoarthritis Initiative (OAI) reported that one quarter of people with knee OA experienced concurrent foot pain with the majority (55%) reporting pain in both feet, and knee OA patients with foot/ankle symptoms reported worse scores on all WOMAC subscales including the total score, worse health outcomes and poorer physical function compared with those without foot/ankle symptoms[303]. In our study, over half the patients (51.8%) reported foot pain at baseline and patients with foot pain had lower quality of life and higher rates of depression, which was similar to previous studies. One study reported that foot/ankle symptoms in either or both feet significantly increased the odds of developing knee symptoms and symptomatic radiographic knee OA in people at risk of the disease[311]. Additionally, in patients with symptomatic radiographic knee OA, the presence of foot/ankle symptoms was associated with increased risk of knee pain over four years[312]. Owing to the coexistent relationships between foot/ankle symptoms and knee OA, more attention should be paid in management of foot pain in OA patients.

Although there is a growing body of evidence suggesting that a low level of vitamin D is associated with chronic pain, no clinical study has been conducted to explore the effect of vitamin D supplementation on foot pain. In addition, studies examining whether vitamin D supplementation is beneficial on other musculoskeletal pains are limited and found contradictory results[167 305 313-315], mainly due to variations in participants, outcome measures, sample size, vitamin D dose and follow-up time. A recent secondary analysis of an RCT study with large sample suggested long-term monthly 100,000 IU vitamin D supplementation did not improve pain scores or reduce analgesic dispensing in the general population[315]. Similarly, a

Cochrane review also concluded that a large beneficial effect of vitamin D on chronic painful conditions across different sites was unlikely[305]. However, in this secondary analysis of the VIDEO study, we investigated the effect of vitamin D supplementation on foot pain and found a reduction in MFPDI scores in knee OA patients after 24 months compared with the placebo group, particularly in patients with disabling foot pain at baseline. There are a number of reasons as to why our results vary from other studies. Our participants were selected to have low baseline vitamin D levels, we specifically examined foot pain using a validated measure, our vitamin D dose was 50,000IU per month, and our duration of treatment was 2 years. Further clinical trials will be needed to determine whether vitamin D supplementation is beneficial for foot pain in patients with knee OA. Consistently, treatment with vitamin D could improve knee pain assessed using VAS. However, vitamin D supplementation did not improve the knee pain assessed using the WOMAC scale. The reasons underlying these are unclear but may reflect variations in measurements. Further clinical trials are required to confirm the findings.

Previous studies have found that low levels of vitamin D are associated with chronic pain[167 172 173], but there is no previous work linking vitamin D deficiency to foot pain. One population-based, cross-sectional study of 958 older adults, found that a lower level of vitamin D was not related with foot pain, but was related with back pain[316]. In contrast, our study showed that patients who maintained vitamin D sufficiency had significantly decreased MFPDI scores compared with those did not maintain vitamin D sufficiency between month 3 and 24 suggesting that correction of vitamin D deficiency might reduce foot pain over time.

Several potential mechanisms such as bone demineralization, muscle weakness and pain dysregulation may link vitamin D deficiency to musculoskeletal pain. Vitamin D can modulate a number of inflammatory pathways[317], which are associated with pain sensitization. Low vitamin D level can activate proinflammatory cytokine proliferation, thus alter sensitization of peripheral and central pathways through nociceptive inflammation processing[318 319], which may be an important contributor to clinical symptoms of knee OA[320]. Even though the underlying mechanisms between vitamin D deficiency and foot pain are still unclear and further investigations are needed.

There are several potential limitations in our study. First, this is a post hoc analysis in which foot pain was the secondary outcome in the original protocol. Second, nearly half of patients reported some foot pain at baseline, only 23.7% of patients had disabling foot pain according

to the definition we used in this study. Even though the MFPDI score increased less after vitamin D supplementation in those without disabling foot pain, the clinically significant difference of MFPDI score is unknown. Third, 62% of patients in placebo group reached sufficient vitamin D levels after 24 months' follow-up, which might due to seasonal change, outdoor physical activity or other reasons that may underestimate any benefit of vitamin D. In support of this, beneficial effects of vitamin D were also found in MFDPI scores after patients were divided into consistently and not consistently sufficient vitamin D group.

In summary, this is the first study to show that vitamin D supplementation and maintaining sufficient vitamin D levels reduce foot pain over two years in patients with symptomatic knee OA. Vitamin D supplementation and maintaining sufficient vitamin D level may improve foot pain in knee OA patients.

Chapter 9 Summary and future directions

OA is a highly prevalent, painful, and disabling joint disease. It is often co-existing with chronic comorbidities. OA has been described as “a serious disease” with no proven effective intervention, having significant impacts and constituting major challenges on individuals and public health system globally. Furthermore, co-occurrence with comorbidity in OA patients is also projected to increase difficulties for management and increase disease burden. Thus, the prevention, retarding disease progression and management of symptoms and comorbidity are of great importance. Identifying risk factors and management of modifiable risk factors could be a key for prevention and intervention. Vitamin D deficiency has been proved as a modifiable risk factor for OA and its comorbidity. Therefore, correcting vitamin D deficiency may have promising beneficial effects on OA and comorbidity. This thesis has focused on the effect of vitamin D on OA and comorbidity, as well as risk factors for comorbidity in OA patients. It has presented several novel findings from a randomised controlled clinical trial (VIDEO) in participants with symptomatic knee OA and vitamin D deficiency at baseline.

9.1 Summary of findings

Chapter 4 is the first describing the differences in disease progression and knee symptoms among people with knee OA by vitamin D status over time. 340 participants who completed the VIDEO study were classified as consistently insufficient [serum 25(OH)D≤50nmol/l at month 3 and 24, n=45], fluctuating [25(OH)D>50nmol/l at either point, n=68] and consistently sufficient [25(OH)D>50nmol/l at month 3 and 24, n=226] vitamin D groups. The consistently sufficient group had significantly decreased loss of tibial cartilage volume (β: 2.1%, 95% CI: 0.3% to 3.9%) and less increase in effusion-synovitis volume (β: -2.5ml, 95% CI: -4.7 to -0.2ml) compared to the consistently insufficient group in multivariable analyses. In addition, participants who maintained sufficient serum 25(OH)D levels over two years were associated with more improvement of WOMAC physical function (β: -94.2, 95% CI, -183.8 to -4.5) than those with persistent vitamin D insufficiency. However, no significant differences in changes in cartilage defects, bone marrow lesions or knee pain between groups were found. These results suggest beneficial effects of maintaining vitamin D sufficiency on cartilage loss, effusion-synovitis and physical function in people with symptomatic knee OA.

Low-grade systemic inflammation triggered by abnormally inflammatory or metabolic biomarkers has been implicated in the OA pathogenesis. Evidence has suggested vitamin D may modify OA disease progression through inhibition of inflammation. Chapter 5 explores whether vitamin D supplementation affected serum inflammatory and metabolic biomarkers and whether variation in vitamin D status over two years was associated with the change in biomarkers in patients with knee OA and vitamin D deficiency. 200 participants from one site (94 from placebo group and 106 from vitamin D group) from the VIDEO study were randomly selected for measurement of serum levels of inflammatory and metabolic biomarkers at baseline and 24 months. Additionally, participants were classified into two groups according to serum 25-hydroxyvitamin D [25(OH)D] levels at month 3 and 24: 1) not consistently sufficient [25(OH)D≤50nmol/l at either month 3 or 24, N= 61], and 2) consistently sufficient [25(OH)D>50nmol/l at both month 3 and 24, N= 139]. Compared with placebo, monthly 50,000 IU vitamin D supplementation had no significant effect on change in serum hs-CRP, IL-6, IL-8, IL-10, leptin, adiponectin, resistin, adipsin and apelin. Being consistently vitamin D sufficient over 2 years was also not associated with changes in these biomarkers compared to not being consistently sufficient. Vitamin D supplementation and maintaining vitamin D sufficiency did not alter serum levels of inflammatory and metabolic biomarkers over 2 years

in knee OA patients who were vitamin D insufficient, suggesting they may not affect systemic inflammation in knee OA patients.

Depression and depressive symptoms are common among individuals with OA. The potential therapeutic potential of vitamin D supplementation for depression has been highlighted. Chapter 6 investigates the effect of vitamin D supplementation and maintaining sufficient serum vitamin D on depressive symptoms in patients with knee OA patients. Participants with symptomatic knee OA and vitamin D deficiency were enrolled in the VIDEO study and received 50,000IU vitamin D_3 (N= 209) or placebo (N= 204) monthly for 24 months. Depressive symptoms were assessed using the PHQ-9 questionnaire. PHQ-9 scores improved more in the vitamin D treatment group (β: -0.45, 95% CI: -0.84 to -0.07) compared to the placebo group (β: 0.21, 95% CI: -0.19 to 0.61) (P for difference = 0.02) and in the participants who maintained vitamin D sufficiency between month 3 and 24 (β: -0.44, 95% CI: -0.88 to -0.00) compared to those who did not (β: 0.40, 95% CI: -0.18 to 0.97) (P for difference = 0.02) over 24 months. These findings show that vitamin D supplementation and maintaining adequate vitamin D levels over 24 months may be beneficial for depressive symptoms in patients with knee OA.

Chapter 7 describes if the demographic and clinical factors were associated with the prevalence and incidence of depression and explored the temporal relationship between depression and joint symptoms in patients with symptomatic knee OA. The prevalence and the incidence of depression was 25.4% and 11.2%, respectively. At baseline, having a higher BMI, greater scores of WOMAC pain (PR: 1.05, 95% CI:1.03, 1.07), dysfunction (PR: 1.02, 95% CI:1.01, 1.02) and stiffness (PR: 1.05, 95% CI: 1.02, 1.09), a lower education level, having one more comorbidity and having two or more site pain were significantly associated with a higher prevalence of depression. Over 24 months, being female, having a higher WOMAC pain (RR: 1.04, 95% CI: 1.00, 1.08) and dysfunction score (RR: 1.01, 95% CI: 1.00, 1.02) and having two or more site pain were significantly associated with a higher incidence of depression. In contrast, baseline depression was not associated with changes in knee joint symptoms over 24 months. Overall, knee OA risk factors and symptoms and co-existing with multi-site pain are associated with the prevalence and development of depression, suggesting the importance of managing common OA risk factors and symptoms for prevention and treatment of depression in patients with knee OA.

Chapter 8 describes the effect of vitamin D supplementation and maintaining sufficient serum vitamin D on foot pain in patients with knee OA patients. Foot pain severity was assessed using the MFPDI index. In this sample, 51.8% of participants reported they had foot pain in patients with knee OA and vitamin D deficiency. MFPDI scores decreased slightly in the vitamin D group (β: -0.03, 95% CI: -0.80 to 0.74) while worsened in the placebo group (β: 1.30, 95% CI: 0.51 to 2.09), and there were significant differences in change of MFPDI scores between groups in the mixed-effect model after including all time points adjusted for age, sex and BMI (-0.03 in vitamin D group vs. 1.30 in placebo group; between-group difference, β: -1.32, 95% CI: -2.43 to -0.22, P= 0.013). Post-hoc analyses comparing participants who maintained sufficient vitamin D with who did not maintain vitamin D sufficiency showed similar results in change of MFPDI score over 2 years (2.06 in insufficient vitamin D group vs. 0.08 in maintaining sufficient vitamin D group; between-group difference, β: -1.98, 95%CI: -3.27 to -0.68, P= 0.003). These suggest that vitamin D supplementation and maintaining adequate vitamin D levels over 24 months had beneficial effects on foot pain deterioration in OA patients.

In summary, the findings from this thesis indicate that depression and foot pain are common comorbidities in patients with knee OA. Using multi-disciplinary approaches to manage common OA risk factors, chronic pain and joint dysfunction may be beneficial for the prevention and management of depression in knee OA patients. More importantly, vitamin D supplementation and/or maintaining vitamin D sufficiency may not affect systemic inflammation but may have beneficial effects on cartilage loss, effusion-synovitis, physical function and comorbidities including depression and foot pain in patients with knee OA. Recommendations for the future direction of each chapter are provided in the following.

9.2 Future directions

Chapter 4 uses the actual serum vitamin D levels to overcome the potentially diluting effect from those who achieved sufficient vitamin D levels in the placebo group during the trial. It has confirmed the importance of maintaining vitamin D sufficiency in symptomatic knee OA patients. However, for the nature of post hoc study and previous randomised controlled trials had some major limitations, the effect of correcting vitamin D deficiency on OA needs to be confirmed by further clinical trials with good designs and high quality. Adequate sample size, excluding participants with severe disease severity, enrolling participants with defined vitamin D deficiency, enough treatment duration, and using sensitive techniques to measure OA

outcomes are essential keys for further clinical trials[147]. Of importance, how to keep the vitamin D in low levels in the placebo group throughout the trial deserves a careful pondering. The Mendelian randomised studies has promising to answer this question[321].

Chapter 5 has demonstrated that vitamin D supplementation and maintaining sufficient vitamin D status may not have effects on systemic inflammation in knee OA patients. The reasons underlying these are unclear and it may also be related to the studied OA population which was not selected as being with an inflammatory phenotype. In another post-hoc analysis of the VIDEO study, we reported that vitamin D supplementation relieved the progression of effusion-synovitis in patients with an inflammatory OA phenotype[151]. These indicate that vitamin D supplementation would have effects on local rather than systemic inflammation in inflammatory OA. In future studies, the effect of vitamin D on local rather than systemic inflammation needs to be examined in knee OA patients with an inflammatory phenotype. Moreover, the potential mechanisms linking vitamin D deficiency to inflammation in the pathogensis of OA need to be explored further.

Chapter 6 and 8 investigate the effect of vitamin D supplementation and maintaining vitamin D sufficiency on depressive symptoms and foot pain over 24 months in patients with knee OA and vitamin D deficiency. These results show the potentially important role of vitamin D on OA comorbidities. However, due to a lack of information about the clinically important difference for patients with milder depressive symptoms and foot pain currently, it remains unclear whether the statistically significant improvements in depressive symptoms and foot pain we reported in this study are clinically important. Therefore, more clinical studies are needed in the future to examine the clinical significance. Moreover, the prevalence of depression was 25.4% and of foot pain was 51.8% in the VIDEO sample. Further RCTs are required to examine the effects of depression or foot pain in patients with both knee OA and depression or foot pain. Most of important, why vitamin D supplementation improved foot pain but did not improve knee pain has to be explored and illuminated in future study. Whether measurement of knee pain is interference in statistically significant results and whether vitamin D only have beneficial effect on specific pain has to be answered.

Chapter 7 provide empirical evidence that management of common OA risk factors, chronic pain and joint dysfunction may be beneficial for preventing and managing depression in knee OA patients. However, whether management of chronic pain and joint dysfunction has benefits

on depression or prevents depression occurring and whether baseline depression severity could moderate the intervention effects on joint symptoms are required to fully clarify. More importantly, having comorbidities in OA patients may contribute to increased challenges of treating OA patients. Therefore, treating each patient individually by a multi-disciplinary approach should be considered.

APPENDIX I Questionnaires

WOMAC Questionnaire

Menzies Research Institute

MONASH University

Site Number: ☐

Randomisation Code: ☐☐☐☐

Initials: ☐☐☐

Visit Number: ☐

Date: ☐☐ / ☐☐ / ☐☐☐☐

VIDEO Study: WOMAC

Instructions: Please read carefully

Please answer all questions to the best of your ability (leave blank if unknown).

Your answers will be completely confidential.

Please place a mark on the line to rate the following today for your knee

Examples	**None**	**Severe**
Example of no pain	|	
Example of severe pain		|

			Office use only
1. Referring to your **knees only** how much **pain** do you experience when	**None**	**Severe**	
a. Walking on a flat surface			
b. Going up and down stairs			
c. At night while in bed			
d. Sitting or lying			
e. Standing upright			
2. Referring to your **knees only** how much **stiffness** do you experience	**None**	**Severe**	
a. After first awakening			
b. Later in the day			
3. Referring to your **knees only** how much **functional deficit** do you experience when	**None**	**Severe**	
a. Descending stairs			
b. Ascending stairs			
c. Rising from bed			
d. Rising from sitting			
e. Putting on socks			
f. Taking off socks			
g. Bending to the floor			
h. Lying in bed			

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Question 3 continued

	None — Severe	Office use only
i. Walking on flat surface		
j. Getting in/out of the bath		
k. Standing		
l. Sitting		
m. Getting in/out of the car		
n. Getting on/off the toilet		
o. Heavy domestic chores		
p. Light domestic chores		
q. Shopping		

Patients Health Questionnaire

Menzies Research Institute

MONASH University

VIDEOStudy

Site Number: ☐

Randomisation Code: ☐☐☐☐

Initials: ☐☐☐

Visit Number: ☐

Date: ☐☐ / ☐☐ / ☐☐☐☐

VIDEO Study: Personal Health Questionnaire Depression Scale

Instructions: Please read carefully

Please answer all questions to the best of your ability (leave blank if unknown).

Your answers will be completely confidential.

Indicate your response by filling in the circle next to the most appropriate answer.

Example

Shade Circles Like This	●
Not Like This	⊗ or ✓
Cross Out Mistakes Like This	⊗●

Or by writing clearly using the boxes where provided.

Please use BLOCK LETTERS where required. e.g H O B A R T ☐ ☐

Cross out any mistakes & write correct answer just below the relevant boxes.

Please use a black or blue pen if possible

Over the **last 2 weeks**, how often have you been bothered by any of the following problems *(Fill in **one** circle on each line)*

How often during the past 2 weeks were you bothered by ...

	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things.	O	O	O	O
2. Feeling down, depressed or hopeless.	O	O	O	O
3. Trouble falling or staying asleep, or sleeping.	O	O	O	O
4. Feeling tired or having little energy.	O	O	O	O
5. Poor appetite or overeating.	O	O	O	O
6. Feeling bad about yourself, or that you are a failure, or have let yourself or your family down.	O	O	O	O
7. Trouble concentrating on things, such as reading the newspaper or watching television.	O	O	O	O
8. Moving or speaking so slowly that other people could have noticed. Or the opposite - being so fidgety or restless that you have been moving around a lot more than usual.	O	O	O	O
9. Thoughts that you would be better off dead, or of hurting yourself in some way.	O	O	O	O

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Manchester Foot Pain and Disability Index questionnaire

Menzies Research Institute

MONASH University

VIDEOStudy

Site Number: ☐

Randomisation Code: ☐☐☐☐

Initials: ☐☐☐

Visit Number: ☐

Date: ☐☐ / ☐☐ / ☐☐☐☐

VIDEO Study: Manchester Foot Pain and Disability Index

Instructions: Please read carefully

Please answer all questions to the best of your ability (leave blank if unknown).

Your answers will be completely confidential.

Indicate your response by filling in the circle next to the most appropriate answer

Example

Shade Circles Like This	●
Not Like This	⊗ or ✓
Cross Out Mistakes Like This	⊗●

Or by writing clearly using the boxes where provided.

Please use BLOCK LETTERS where required e.g H O B A R T ☐ ☐

Cross out any mistakes & write correct answer just below the relevant boxes
Please use a black or blue pen if possible

Below are some statements about problems people have because of **pain in their feet**.
For each statement indicate if this has applied to you during the **past month**. If so, was this only on some days or on most or every day in the past month?

Because of pain in my feet:	None of the time	On some days	On most / every day/s
I avoid walking outside at all	O	O	O
I avoid walking long distances	O	O	O
I don't walk in a normal way	O	O	O
I walk slowly	O	O	O
I have to stop and rest my feet	O	O	O
I avoid hard or rough surfaces when possible	O	O	O

Because of pain in my feet:	None of the time	On some days	On most days / every day
I avoid standing for a long time	O	O	O
I catch the bus or use the car more often	O	O	O
I need help with housework / shopping	O	O	O
I still do everything but with more pain or discomfort	O	O	O
I get irritable when my feet hurt	O	O	O
I feel self-conscious about my feet	O	O	O
I get self-conscious about the shoes I have to wear	O	O	O
I have constant pain in my feet	O	O	O
My feet are worse in the morning	O	O	O
My feet are more painful in the evening	O	O	O
I get shooting pains in my feet	O	O	O

Because of pain in my feet:	None of the time	On some days	On most / every day/s
I am unable to carry out my previous work	O	O	O
I no longer do all my previous activities (sport, dancing, hill-walking, etc)	O	O	O

Other foot pain questions

Do you suffer from pain in your feet — Yes O No O

If 'Yes', how long have you had foot pain? ☐☐☐ months

Where is your foot pain?

Heel O Arch O Ball of foot O Toes O Whole foot O Other O

Do you have arthritis in your feet? — Yes O No O

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APPENDIX II Published Manuscripts

Maintaining vitamin D sufficiency and OA

CLINICAL RESEARCH STUDY

THE AMERICAN JOURNAL of MEDICINE®

Maintaining Vitamin D Sufficiency Is Associated with Improved Structural and Symptomatic Outcomes in Knee Osteoarthritis

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ABSTRACT

BACKGROUND: The aim of this study was to describe whether maintaining sufficient serum vitamin D levels in people with knee osteoarthritis and baseline vitamin D insufficiency has an association with change in knee structures and symptoms over 2 years.

METHODS: Participants (n = 413, mean age 63.2 years) with symptomatic knee osteoarthritis and vitamin D insufficiency were enrolled in a clinical trial. In all, 340 participants (82.3%) completed the study, with 25-hydroxyvitamin D [25(OH)D] measurements at baseline and months 3 and 24. Participants were classified as consistently insufficient [serum 25(OH)D ≤50 nmol/L at months 3 and 24, n = 45], fluctuating [25(OH)D >50 nmol/L at either point, n = 68), and consistently sufficient [25(OH)D >50 nmol/L at months 3 and 24, n = 226] groups. Knee cartilage volume, cartilage defects, bone marrow lesions, and effusion-synovitis volume were assessed using MRI at baseline and month 24. Knee symptoms were assessed at baseline and months 3, 6, 12, and 24 using the Western Ontario and McMaster Universities Arthritis Index.

RESULTS: The consistently sufficient group had significantly less loss of tibial cartilage volume (β 2.1%; 95% confidence interval [CI], 0.3%, 3.9%), less increase in effusion-synovitis volume (β −2.5 mL; 95 CI %, −4.7, −0.2 mL), and less loss of Western Ontario and McMaster Universities Arthritis Index physical function (β −94.2; 95% CI, −183.8, −4.5) compared with the consistently insufficient group in multivariable analyses. In contrast, there were no significant differences in these outcomes between the fluctuating and consistently insufficient groups. Changes in cartilage defects, bone marrow lesions, and knee pain were similar between groups.

CONCLUSION: This post hoc analysis suggests beneficial effects of maintaining vitamin D sufficiency on cartilage loss, effusion-synovitis, and physical function in people with knee osteoarthritis.

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KEYWORDS: Knee osteoarthritis; MRI; Post hoc; Vitamin D

Osteoarthritis and vitamin D deficiency are very common conditions worldwide, often co-existing, especially in the aging population.[1,2] Osteoarthritis is a major cause of chronic pain and impaired physical function in older adults and has contributed substantially to an increased economic burden and imposed huge challenges on health systems worldwide.[3,4]

Funding: Australian National Health and Medical Research Council Grant (Project Code 605501).

Conflict of Interest: None.

Authorship: CD has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. SZ and CD designed and carried out data analyses, interpreted the results, and drafted the manuscript. XJ, FC, XW, ZZ, AW, WH, TW, BA, DA, LB, and GJ were involved in collecting the data, designing the data analyses, interpreting the results, and revising the manuscript for important intellectual content. All authors approved the final version for submission.

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0002-9343/$ -see front matter
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The ideal treatment for osteoarthritis is to reduce symptoms and slow disease progression. These may, in turn, reduce the impact of osteoarthritis on patients' mobility and quality of life, with a consequent reduction in the need for joint replacement surgery and health care costs in the long term.[5] In experimental studies, sufficient vitamin D can protect against increased bone turnover and cartilage degradation.[6-8] Although there is increasing epidemiologic evidence suggesting that insufficient serum vitamin D status is associated with the progression of osteoarthritis and worsening in its symptoms, the results have been inconsistent.[9]

CLINICAL SIGNIFICANCE

- The evidence of whether vitamin D supplementation is an effective treatment for osteoarthritis is contradictory.
- Patients who are maintaining vitamin D sufficiency had significantly less loss of tibial cartilage volume, less increase in effusion-synovitis volume, and less loss of Western Ontario and McMaster Universities Arthritis Index physical function compared with those who did not maintain vitamin D sufficiency in patients with symptomatic knee osteoarthritis.
- Maintaining vitamin D sufficiency may have beneficial effects in knee osteoarthritic patients.

In the Vitamin D Effect on Osteoarthritis (VIDEO) randomized, controlled trial we reported that vitamin D supplementation did not prevent tibial cartilage loss or improve knee pain as assessed using the Western Ontario McMaster Osteoarthritis Index (WOMAC) but had significant but small effects on visual analog scale (VAS) knee pain, total WOMAC score, and WOMAC function in post hoc analyses in participants with symptomatic knee osteoarthritis and insufficient vitamin D levels.[10] Although the level of 25-hydroxyvitamin D [25(OH)D] increased much more in the vitamin D group (40.6 nmol/L) than in the placebo group (6.7 nmol/L) over 2 years, 62% participants in the placebo group still reached a sufficient level of serum 25(OH)D (>50 nmol/L) at month 24 (unpublished data). Thus the high proportion of participants achieving sufficient 25(OH)D levels in the placebo group may have masked the beneficial effects of vitamin D supplementation.

Therefore, we conducted a post hoc analysis of the VIDEO study to describe whether maintaining sufficient serum vitamin D levels in people with knee osteoarthritis and baseline vitamin D insufficiency had an association with change in knee structures and symptoms over 2 years.

METHODS

Participants

This study was a post hoc analysis of the VIDEO study. VIDEO was a multicenter, randomized, double-blind, placebo-controlled clinical trial in Tasmania and Victoria, Australia, which aimed to evaluate the effect of vitamin D supplementation over 2 years on knee pain and knee cartilage volume in people with symptomatic knee osteoarthritis combined with low 25(OH)D levels. Trial design and inclusion and exclusion criteria have been described in the published protocol.[11] Participants had symptomatic knee osteoarthritis (assessed using American College of Rheumatology criteria[12]) at least for 6 months and had pain of >20 mm on a 100-mm VAS, with low levels of 25(OH) D (between 12.5 and 60 nmol/L). Participants with severe radiographic changes (grade 3 of the Altman and Gold Atlas[13]), severe knee pain on standing (>80 mm on a 100-mm VAS), contraindications to magnetic resonance imaging (MRI), rheumatoid or psoriatic arthritis, lupus, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, conditions affecting oral drug absorption, anticipated knee or hip surgery within the next 2 years, history of significant trauma of knees (eg, arthroscopy or injury to ligaments or menisci within 1 year preceding the study), and history of taking vitamin D or an investigational drug within the last 30 days were excluded.[11] A total of 413 participants with a mean age of 63.2 years were included, and 340 completed the study, with serum 25(OH)D levels measured at months 3 and 24.

Vitamin D Measurement and Groups

Serum 25(OH)D was measured at baseline and months 3 and 24, using direct competitive chemiluminescent immunoassays (DiaSorin, Saluggia, Italy). The intra-assay and interassay coefficients of variation were 3.2% and 6.0%, respectively.[10] Serum 25(OH)D levels of ≤50 nmol/L were defined as vitamin D deficient, and levels >50 nmol/L were defined as vitamin D sufficient.[14,15] Participants for the present analysis were classified into 3 groups based on the levels of 25(OH)D at months 3 and 24: consistently insufficient [serum 25(OH)D ≤50 nmol/L at both months 3 and 24], fluctuating [serum 25(OH)D >50 nmol/L at either point], and consistently sufficient [serum 25(OH)D >50 nmol/L at both months 3 and 24] vitamin D groups.

Assessment of Knee Structural Changes

Magnetic resonance imaging scans of the study knee were obtained according to a standardized protocol using a 1.5-T whole-body MRI unit with a commercial transmit–receive extremity coil at baseline and 2 years. The sequences used for cartilage volume assessment were sagittal fat saturated

T1-weighted spoiled gradient echo. Cartilage defects, bone marrow lesions, and effusion-synovitis volume were assessed using T2-weighted/proton density–weighted fast spin echo sequences. Magnetic resonance images were assessed by trained readers blinded to treatment allocations, according to methods described previously.[11,16,17]

Cartilage volume was determined using the previously described image processing techniques.[11] The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled using bilinear and cubic interpolation for final 3-dimensional rendering using OsiriX imaging software (32-bit version 5.9, Pixmeo SARL, Bernex, Switzerland). The coefficient of variation was 2.1% for medial tibia and 2.2% for the lateral tibia.

Cartilage defects (0-4) were graded using a modification of the Outerbridge classification system at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, with details described in the protocol.[18] A total score of the tibiofemoral compartment was calculated as the total of 2 subregional scores (medial tibial and femoral, lateral tibial and femoral, 0-8). Intraobserver reliability expressed as an intraclass correlation coefficient ranged from 0.77 to 0.94.

Bone marrow lesions, defined as discrete areas of increased signal adjacent to the subcortical bone, were measured using a modification of the classification system of Whole-Organ Magnetic Resonance Imaging Score (0 = none, 1 = ≤25% of the subregion, 2 = 25%-50%, and 3 = ≥50%).[17] A total score of the tibiofemoral compartment was calculated as the total of 12 subregional scores (0-36). The intraclass correlation coefficient of this bone marrow lesions measurement ranged from 0.93 to 0.98.

Effusion-synovitis volume at 4 regions (suprapatellar pouch, central portion, posterior femoral recess, and subpopliteal recess) were isolated from the total volume selecting each region of interest according to the intra-articular fluid-equivalent signal on a section-by-section basis and then resampled by means of bilinear and cubic interpolation for final 3-dimensional rendering using OsiriX software.[19,20] The intraclass correlation coefficient ranged from 0.96 to 0.97.

Change in cartilage volume and effusion-synovitis volume were calculated as follows:

$$\text{Absolute change(mL)} = (\text{follow} - \text{up volume}) - (\text{baseline volume});$$

$$\text{Percentage change per annum(\%p.a.)} = [(\text{absolute change})/(\text{baseline volume})]/(\text{time interval between 2 scans in years}) \times 100.$$

Change in cartilage defects and bone marrow lesions were calculated as follows:

$$\text{Cartilage defects change} = (\text{follow} - \text{up cartilage defects}) - (\text{baseline cartilage defects});$$

$$\text{Bone marrow lesions change} = (\text{follow} - \text{up bone marrow lesions}) - (\text{baseline bone marrow lesions}).$$

Assessment of Symptomatic Changes

Knee symptoms were assessed at baseline and months 3, 6, 12, and 24 using WOMAC and the VAS.[21,22] The total WOMAC score (0-2400) is the sum of subscale scores including pain (0-500), stiffness (0-200), and physical function (0-1700).

Anthropometrics and Questionnaires

Height was measured to the nearest 0.1 cm (with shoes removed) using a stadiometer (Leicester Height Measure, Invicta Plastics, Leicester, United Kingdom). Weight was measured to the nearest 0.1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307, Heine, Dover, NH). Body mass index (kg/m^2) was calculated. We also recorded use of nonsteroidal anti-inflammatory drugs in VIDEO.

STATISTICAL ANALYSIS

The one-way analysis of variance or Kruskal-Wallis rank tests were used to compare differences in baseline characteristics (age, sex, body mass index, cartilage volume, cartilage defects, bone marrow lesions, effusion-synovitis volume, VAS scores, and WOMAC scores) among the 3 vitamin D groups. To take into account missing data, we assumed data were missing at random and used a weighted estimating equation method.[23,24] We estimated the probability of a participant remaining in the study during follow-up by fitting a logistic regression model using the baseline characteristics age, sex, body mass index, and level of 25-(OH)D as predictors, for which complete data were available. In subsequent analyses, completed cases were weighted by the inverse of their estimated probabilities of being observed. Univariable and multivariable linear regressions were used to examine the difference in the changes in cartilage volume, cartilage defects, bone marrow lesions, and effusion-synovitis volume between vitamin D groups before and after adjustment for age, sex, body mass index, and change in season of blood sampling. The difference in changes of symptoms between different

vitamin D groups was analyzed using a repeated-measures mixed model with terms for age, sex, body mass index, and season of blood sampling. The correlation between the repeated measures was addressed by using individual participant identification as a random effect. The differences between groups were further adjusted for use of nonsteroidal anti-inflammatory drugs. We used Stata 12.0 for Windows (StataCorp, College Station, Tex) for all analyses. A P value $<.05$ (2-tailed) was regarded as statistically significant.

RESULTS

Participants and Groups

A total of 413 participants (mean age 63.2 years, 50% women) with symptomatic knee osteoarthritis and low vitamin D levels were enrolled in the VIDEO study from June 2010 to December 2011. Of these, 340 participants (82.3%) completed the study with 25(OH)D measurements at months 3 and 24. At baseline, participants who did not complete the study were more likely to be female and had lower tibial cartilage volume than those who completed, but there were no other significant differences in baseline characteristics between these groups.[10] Baseline characteristics of participants in the 3 vitamin D status groups are shown in Table 1. Forty-six participants were classified as consistently insufficient (mean age 62.6 years, 52.2% female), 68 as fluctuating (mean age 62.9 years, 55.9% female), and 226 as consistently sufficient (mean age 63.5 years, 43.4% female) vitamin D groups. There were no significant differences in baseline characteristics among groups.

Change in Knee Joint Structures

There was a dose–response relationship between the status of serum vitamin D and change in total tibial cartilage volume (Figure 1). Participants with consistently sufficient vitamin D experienced significantly less loss of total tibial cartilage volume per year than participants with consistently insufficient vitamin D (Figure 1, Table 2). The differences between these 2 groups and among the 3 groups remained significant after adjustment for age, sex, body mass index, and change in season of blood sampling. A similar pattern was seen for medial and lateral tibial cartilage volume, but the trend did not reach statistical significance in adjusted analyses (all $P \leq .10$) (data not shown). Similarly, participants with consistently sufficient vitamin D had significantly fewer increases in effusion-synovitis volume (absolute and percentage per year) compared with participants with consistently insufficient vitamin D (Figure 2, Table 3). The differences between these 2 groups and among the 3 groups remained significant after adjustment for age, sex, body mass index, and season of blood sampling (Table 3). In contrast, there were no significant differences in change in cartilage volume or effusion-synovitis volume between the fluctuating and consistently insufficient vitamin D groups. Additionally, there were no significant differences in changes in total cartilage defects and bone marrow lesions between and among groups (Tables 2 and 3). We

Table 1 Baseline Characteristics in Groups with Different Serum Vitamin D Concentrations

Characteristic	Consistently Insufficient (n = 46)	Fluctuating (n = 68)	Consistently Sufficient (n = 226)	P value
Sex (female), n (%)	24 (52.2)	38 (55.9)	98 (43.4)	.15
Age (y)	62.6 (8.0)	62.9 (6.1)	63.5 (7.2)	.68
Body mass index (kg/m^2)	30.6 (4.6)	29.0 (4.5)	29.4 (4.9)	.21
Cartilage volume (cm^3)				
Lateral tibial	2.0 (0.7)	2.0 (0.6)	2.1 (0.7)	.32
Medial tibial	1.4 (0.5)	1.5 (0.5)	1.6 (0.5)	.12
Total tibial	3.4 (1.1)	3.5 (0.9)	3.7 (1.1)	.15
Tibiofemoral dartilage defects, scores (0-8)				
Lateral	4.4 (1.8)	4.4 (1.7)	4.3 (1.9)	.80
Medial	5.2 (2.0)	4.9 (2.1)	4.7 (2.1)	.29
Tibiofemoral bone marrow lesions, scores (0-18)				
Lateral	0.6 (1.0)	0.9 (1.4)	0.9 (1.4)	.48
Medial	1.7 (2.7)	1.6 (2.6)	1.3 (2.2)	.55
Effusion-synovitis volume (cm^3)	5.7 (5.6)	8.0 (9.5)	8.4 (8.5)	.14
WOMAC score system				
Pain (0-500)	144.2 (99.0)	130.0 (77.4)	133.8 (86.6)	.91
Stiffness (0-200)	66.9 (44.8)	61.5 (39.1)	59.6 (39.4)	.64
Function (0-1700)	524.2 (307.0)	440.7 (269.1)	461.8 (308.5)	.37
Nonsteroidal anti-inflammatory drugs use, n (%)	13 (28.3)	19 (27.9)	70 (31.0)	.86

Values are mean (standard deviation) unless otherwise stated. One-way analysis of variance or Kruskal-Wallis rank test was used for the comparisons.
WOMAC = Western Ontario McMaster Osteoarthritis Index.

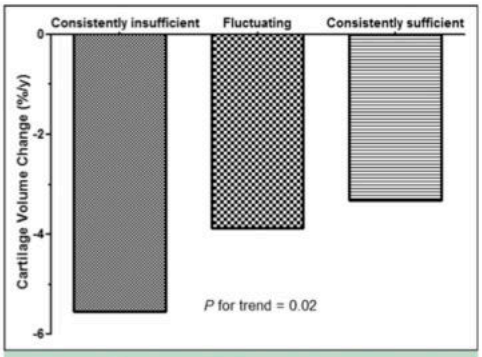

Figure 1 Tibial cartilage volume change (%/y) in knee osteoarthritis patients with or without sufficient serum vitamin D levels over 24 months. Consistently insufficient vitamin D group: serum 25-hydroxyvitamin D [25(OH)D] ≤50 nmol/L at both months 3 and 24; fluctuating vitamin D group: serum 25(OH)D >50 nmol/L at either time point; consistently sufficient vitamin D group: serum 25(OH)D >50 nmol/L at both months 3 and 24.

further adjusted for use of nonsteroidal anti-inflammatory drugs and found that the results remained largely unchanged (data not shown).

Changes in Knee Symptoms

Changes in WOMAC scores over 24 months are shown in Table 4. There were significant differences in WOMAC physical function between consistently sufficient and consistently insufficient vitamin D groups in the mixed-effect models, adjusted for age, sex, body mass index, and change in season of blood sampling (Table 4). Physical function improved time dependently in the consistently sufficient vitamin D group, whereas it fluctuated in the other 2 groups over 24 months (Figure 3). The differences in total WOMAC score and physical function were significant among the 3 groups (Table 4). There were no significant differences in WOMAC pain and stiffness between or among groups (Table 4). After further adjustment for nonsteroidal anti-inflammatory drugs use, the results remained largely unchanged (data not shown).

DISCUSSION

To the best of our knowledge this study is the first describing the differences in disease progression and symptoms among people with knee osteoarthritis by vitamin D status over time. It demonstrated that participants who maintained sufficient serum 25(OH)D levels over 2 years had decreased loss of tibial cartilage volume and less increase in effusion-synovitis volume compared with those who did not. In addition, WOMAC physical function in participants with persistent vitamin D sufficiency improved significantly more than in those with persistent vitamin D insufficiency. However, we did not find significant differences in changes in cartilage defects, bone marrow lesions, or knee pain between groups.

Results from previous randomized, controlled trials have been mixed and do not provide consistent results.[10,25-27] McAlindon et al[26] reported no effect of vitamin D supplementation (vitamin D3 2000 IU/d over 2 years, n = 146) on cartilage volume loss or knee pain in patients with knee osteoarthritis. However, the major limitations of this study were small sample size, the inclusion of participants with both vitamin D sufficiency and insufficiency, and the inclusion of participants with severe disease.[28] Participants with sufficient vitamin D may not benefit from vitamin D supplementation, and patients with severe disease are unlikely to respond to any treatment.[28] Furthermore, a recent trial reported that vitamin D supplementation for 3 years (800 IU/d for 3 years, n = 474) did not slow progression of joint space narrowing or reduce WOMAC pain,

Table 2 Associations Between Maintaining Vitamin D Sufficiency and Changes in Cartilage Volume and Cartilage Defects over 24 Months

	Univariable		Multivariable*	
Variable	β (95% CI)	*P* Value	β (95% CI)	*P* Value
Total tibial cartilage volume change (%/y)				
Consistently insufficient	Reference		Reference	
Fluctuating	1.7 (−0.3, 3.6)	.10	1.5 (−0.5, 3.5)	.15
Consistently sufficient	2.2 (0.4, 4.0)	.02	2.1 (0.3, 3.9)	.03
P for trend		.02		.02
Change in total tibiofemoral cartilage defects				
Consistently insufficient	Reference		Reference	
Fluctuating	−0.3 (−0.8, 0.3)	.40	−0.2 (−0.8, 0.4)	.42
Consistently sufficient	−0.4 (−0.9, 0.1)	.16	−0.4 (−0.9, 0.1)	.15
P for trend		.16		.15

The dependent variables are percentage change in cartilage volume per year or absolute change in cartilage defects over 24 months.
CI = confidence interval.
*Adjusted for age, sex, body mass index, and change in season of blood sampling.

Table 4 Associations Between Maintaining Vitamin D Sufficiency and Changes in Clinical Symptoms over 24 Months

	Change	Multivariable*	
Variable	Mean (95% CI)	β (95% CI)	*P* Value
Total WOMAC score			
Consistently insufficient	−123.2 (−236.1, −10.4)	Reference	
Fluctuating	−111.4 (−194.8, −28.0)	11.8 (−128.5, 152.1)	.87
Consistently sufficient	−240.4 (−298.4, −182.5)	−117.2 (−244.1, 9.6)	.07
P for trend			<.01
Pain			
Consistently insufficient	−34.7 (−66.6, −3.0)	Reference	
Fluctuating	−31.7 (−54.7, −8.8)	3.0 (−36.2, 42.3)	.88
Consistently sufficient	−50.2 (−63.7, −36.6)	−15.4 (−49.9, 19.2)	.38
P for trend			.11
Stiffness			
Consistently insufficient	−11.7 (−25.1, 1.6)	Reference	
Fluctuating	−16.5 (−27.1, −6.0)	−4.7 (−21.7, 12.3)	.59
Consistently sufficient	−20.3 (−26.4, −14.2)	−8.5 (−23.2, 6.1)	.25
P for trend			.17
Function			
Consistently insufficient	−76.0 (−153.6, 1.6)	Reference	
Fluctuating	−61.9 (−118.5, −5.3)	14.8 (−82.6, 112.2)	.77
Consistently sufficient	−170.8 (−212.8, −128.8)	−94.2 (−183.8, −4.5)	.04
P for trend			<.01

Mixed effect model adjusted for age, sex, body mass index, and change in season of blood sampling.
Change in WOMAC score results are generated from mixed models adjusted for age, sex, body mass index, and change in season of blood sampling.
VAS = visual analog scale; WOMAC = Western Ontario McMaster Osteoarthritis Index.
*Adjusted for age, sex, body mass index, and change in season of blood sampling.

with change in cartilage defects in knee osteoarthritis patients, whereas we reported that high serum 25(OH)D levels were associated with decreased knee cartilage volume loss over 2.7 years in older adults.[29]

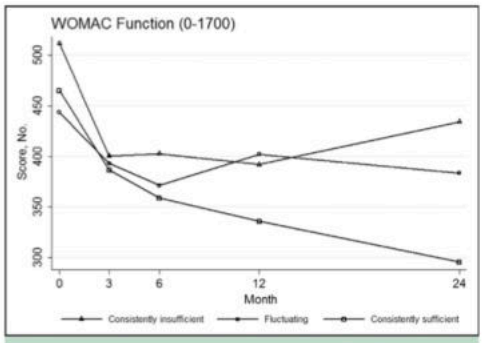

Figure 3 Changes in Western Ontario McMaster Osteoarthritis Index (WOMAC) function in knee osteoarthritis patients with or without sufficient serum vitamin D levels over 24 months. Consistently insufficient vitamin D group: serum 25-hydroxyvitamin D [25(OH)D] ≤50 nmol/L at both months 3 and 24; fluctuating vitamin D group: serum 25(OH)D >50 nmol/L at either time point; consistently sufficient vitamin D group: serum 25(OH)D >50 nmol/L at both months 3 and 24.

The relationship between vitamin D status and joint effusion-synovitis in knee osteoarthritis patients is unknown. In the VIDEO study we recently reported that vitamin D supplementation significantly reduced the increase in effusion-synovitis volume compared with placebo in patients with knee osteoarthritis, particularly those with baseline effusion-synovitis.[31] The results from this study were consistent with this. The present study found that persistent vitamin D sufficiency was associated with improvement in physical function and total WOMAC score, but not with WOMAC pain and WOMAC stiffness. Again, these results were largely consistent with the findings from the VIDEO trial.[10]

Overall, our results suggest that maintaining sufficient serum vitamin D may have a small but beneficial effect on retarding cartilage loss, reducing joint inflammation, and improving physical function in knee osteoarthritis patients. The analyses in the present study have enabled us to use the actual serum vitamin D levels to define groups, which can reduce the potential confounding effect from those who achieved sufficient vitamin D levels in the placebo group during the trial. However, these data were post hoc and data driven and need to be confirmed by further clinical trials. Further, loss of follow-up bias would exist; however, the retention rate in this trial was high (82%), and we used inverse probability weighting to count the impact of the missing values. In addition, we defined consistent vitamin D sufficiency or deficiency using 25(OH)D levels at only months 3 and 24, but the

T1-weighted spoiled gradient echo. Cartilage defects, bone marrow lesions, and effusion-synovitis volume were assessed using T2-weighted/proton density–weighted fast spin echo sequences. Magnetic resonance images were assessed by trained readers blinded to treatment allocations, according to methods described previously.[11,16,17]

Cartilage volume was determined using the previously described image processing techniques.[11] The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled using bilinear and cubic interpolation for final 3-dimensional rendering using OsiriX imaging software (32-bit version 5.9, Pixmeo SARL, Bernex, Switzerland). The coefficient of variation was 2.1% for medial tibia and 2.2% for the lateral tibia.

Cartilage defects (0-4) were graded using a modification of the Outerbridge classification system at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, with details described in the protocol.[18] A total score of the tibiofemoral compartment was calculated as the total of 2 subregional scores (medial tibial and femoral, lateral tibial and femoral, 0-8). Intraobserver reliability expressed as an intraclass correlation coefficient ranged from 0.77 to 0.94.

Bone marrow lesions, defined as discrete areas of increased signal adjacent to the subcortical bone, were measured using a modification of the classification system of Whole-Organ Magnetic Resonance Imaging Score (0 = none, 1 = ≤25% of the subregion, 2 = 25%-50%, and 3 = ≥50%).[17] A total score of the tibiofemoral compartment was calculated as the total of 12 subregional scores (0-36). The intraclass correlation coefficient of this bone marrow lesions measurement ranged from 0.93 to 0.98.

Effusion-synovitis volume at 4 regions (suprapatellar pouch, central portion, posterior femoral recess, and subpopliteal recess) were isolated from the total volume selecting each region of interest according to the intra-articular fluid-equivalent signal on a section-by-section basis and then resampled by means of bilinear and cubic interpolation for final 3-dimensional rendering using OsiriX software.[19,20] The intraclass correlation coefficient ranged from 0.96 to 0.97.

Change in cartilage volume and effusion-synovitis volume were calculated as follows:

$$\text{Absolute change(mL)} = (\text{follow} - \text{up volume}) - (\text{baseline volume});$$

$$\text{Percentage change per annum(\%p.a.)} = [(\text{absolute change})/(\text{baseline volume})]/(\text{time interval between 2 scans in years}) \times 100.$$

Change in cartilage defects and bone marrow lesions were calculated as follows:

$$\text{Cartilage defects change} = (\text{follow} - \text{up cartilage defects}) - (\text{baseline cartilage defects});$$

$$\text{Bone marrow lesions change} = (\text{follow} - \text{up bone marrow lesions}) - (\text{baseline bone marrow lesions}).$$

Assessment of Symptomatic Changes

Knee symptoms were assessed at baseline and months 3, 6, 12, and 24 using WOMAC and the VAS.[21,22] The total WOMAC score (0-2400) is the sum of subscale scores including pain (0-500), stiffness (0-200), and physical function (0-1700).

Anthropometrics and Questionnaires

Height was measured to the nearest 0.1 cm (with shoes removed) using a stadiometer (Leicester Height Measure, Invicta Plastics, Leicester, United Kingdom). Weight was measured to the nearest 0.1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307, Heine, Dover, NH). Body mass index (kg/m^2) was calculated. We also recorded use of nonsteroidal anti-inflammatory drugs in VIDEO.

STATISTICAL ANALYSIS

The one-way analysis of variance or Kruskal-Wallis rank tests were used to compare differences in baseline characteristics (age, sex, body mass index, cartilage volume, cartilage defects, bone marrow lesions, effusion-synovitis volume, VAS scores, and WOMAC scores) among the 3 vitamin D groups. To take into account missing data, we assumed data were missing at random and used a weighted estimating equation method.[23,24] We estimated the probability of a participant remaining in the study during follow-up by fitting a logistic regression model using the baseline characteristics age, sex, body mass index, and level of 25-(OH)D as predictors, for which complete data were available. In subsequent analyses, completed cases were weighted by the inverse of their estimated probabilities of being observed. Univariable and multivariable linear regressions were used to examine the difference in the changes in cartilage volume, cartilage defects, bone marrow lesions, and effusion-synovitis volume between vitamin D groups before and after adjustment for age, sex, body mass index, and change in season of blood sampling. The difference in changes of symptoms between different

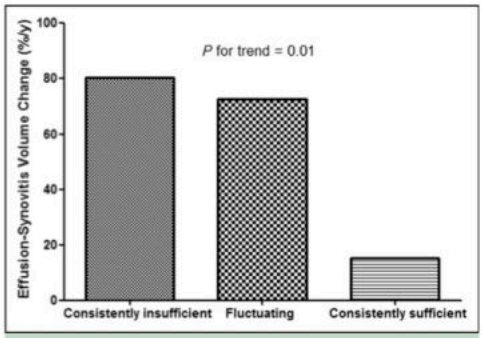

Figure 2 Effusion-synovitis volume change (%/y) in knee osteoarthritis patients with or without sufficient serum vitamin D levels over 24 months. Consistently insufficient vitamin D group: serum 25-hydroxyvitamin D [25(OH)D] ≤50 nmol/L at both months 3 and 24; fluctuating vitamin D group: serum 25(OH)D >50 nmol/L at either time point; consistently sufficient vitamin D group: serum 25(OH)D >50 nmol/L at both months 3 and 24.

stiffness, and function in knee osteoarthritis.[25] However, the researchers used a radiographic measurement as the outcome, which is less sensitive for change. In contrast, another randomised, controlled trial reported that vitamin D supplement at a high dose (60,000 IU/d for 10 days followed by 60,000 IU once per month for 1 year, n = 106) reduced knee pain and improved function in knee osteoarthritis, but this study was limited by its small sample size, short follow-up period, and not examining structural change.[27] The VIDEO study aimed to overcome some of the limitations of previous studies but still had consistent negative results for the primary outcomes. In the secondary analyses we found that the intervention group had small but statistically significant improvements in VAS knee pain and WOMAC function when compared with the placebo group.[10] In addition, 62% of participants achieved a higher level of 25(OH)D (>50 nmol/L) at month 24 in the placebo group (unpublished data), which was thought in part to result from changes in lifestyle (eg, sun exposure), dietary supplementation, supplementation of vitamin D outside the trial, and seasonal variation.

We hypothesized that the high proportion of sufficient vitamin D level in the placebo group might dilute a beneficial effect of vitamin D supplementation. Thus we performed a post hoc analysis to examine whether maintaining sufficient vitamin D levels over time was associated with beneficial effects on joint structural and symptomatic changes in knee osteoarthritis patients in VIDEO study. Those patients who maintained sufficient serum vitamin D levels over 2 years had less loss of total tibial cartilage volume per year than those who did not. Furthermore, there was a dose–response relationship between the status of serum vitamin D (consistently insufficient, fluctuating, and consistently sufficient) and change in total tibial cartilage volume. In contrast, changes in cartilage defects and bone marrow lesions were not significantly different between and among groups. These results were largely consistent with the findings from 2 high-quality cohort studies.[29,30] Felson et al[30] reported that low 25(OH)D status was not associated

Table 3 Association Between Maintaining Vitamin D Sufficiency and Changes in Bone Marrow Lesions and Effusion-Synovitis Volume over 24 Months

	Univariable		Multivariable*	
Variable	β (95% CI)	*P* Value	β (95% CI)	*P* Value
Change in total tibiofemoral bone marrow lesions				
Consistently insufficient	Reference		Reference	
Fluctuating	0.6 (−0.5, 1.6)	.28	0.6 (−0.5, 1.6)	.30
Consistently sufficient	0.5 (−0.4, 1.4)	.25	0.5 (−0.4, 1.4)	.25
P for trend		.36		.33
Effusion-synovitis absolute volume change (mL)				
Consistently insufficient	Reference		Reference	
Fluctuating	0.5 (−2.8, 3.8)	.77	0.7 (−2.5, 3.9)	.66
Consistently sufficient	−2.4 (−4.5, −0.2)	.03	−2.5 (−4.7, −0.2)	.03
P for trend		.01		<.01
Effusion-synovitis volume change (%/y)				
Consistently insufficient	Reference		Reference	
Fluctuating	−9.5 (−118.4, 99.3)	.86	2.2 (−112.2, 116.6)	.97
Consistently sufficient	−69.5 (−133.4, −5.6)	.03	−61.8 (−121.9, −1.7)	.04
P for trend		.01		.01

The dependent variables are absolute change in bone marrow lesions or percentage change per year/absolute change in effusion-synovitis volume over 24 months.
CI = confidence interval.
*Adjusted for age, sex, body mass index, and change in season of blood sampling.

course of 25(OH)D levels between these measurements was unknown.

CONCLUSIONS

This post hoc analysis suggests beneficial effects of maintaining vitamin D sufficiency on cartilage loss, effusion-synovitis, and physical function in people with symptomatic knee osteoarthritis.

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Vitamin D and inflammation in OA

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Vitamin D supplementation and inflammatory and metabolic biomarkers in patients with knee osteoarthritis: *post hoc* analysis of a randomised controlled trial

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Abstract

The aim of this study was to determine whether vitamin D supplementation and maintaining vitamin D sufficiency are associated with changes in inflammatory and metabolic biomarkers in patients with knee osteoarthritis (OA) and vitamin D deficiency. A total of 413 participants with symptomatic knee OA and vitamin D deficiency were enrolled in a randomised, placebo-controlled trial and received 1·25 mg vitamin D_3 or placebo monthly for 24 months across two sites. In this *post hoc* analysis, 200 participants from one site (ninety-four from the placebo group and 106 from the vitamin D group; mean age 63·1 (SD 7·3) years, 53·3 % women) were randomly selected for measurement of serum levels of inflammatory and metabolic biomarkers at baseline and 24 months using immunoassays. In addition, participants were classified into two groups according to serum 25-hydroxyvitamin D (25(OH)D) levels at months 3 and 24: (1) not consistently sufficient (25(OH)D ≤ 50 nmol/l at either month 3 or 24, *n* 61), and (2) consistently sufficient (25(OH)D > 50 nmol/l at both months 3 and 24, *n* 139). Compared with placebo, vitamin D supplementation had no significant effect on change in serum high-sensitive C-reactive protein, IL-6, IL-8, IL-10, leptin, adiponectin, resistin, adipsin and apelin. Being consistently vitamin D sufficient over 2 years was also not associated with changes in these biomarkers compared with not being consistently sufficient. Vitamin D supplementation and maintaining vitamin D sufficiency did not alter serum levels of inflammatory and metabolic biomarkers over 2 years in knee OA patients who were vitamin D insufficient, suggesting that they may not affect systemic inflammation in knee OA patients.

Key words: **Vitamin D: Inflammation: Biomarkers: Knee osteoarthritis**

Osteoarthritis (OA) is a common chronic joint disease associated with increased morbidity and disability risk and contributing to an enormous financial burden on healthcare systems worldwide[1]. In recent years, the pathophysiologic concept of OA has been changed from a degenerative joint disorder to a more complex concept involving multiple aetiologies and pathogeneses[2]. Inflammation is intricately linked to the aetiology of OA and has been implicated in the pathogenesis of OA[3]. Experimental and observational studies have demonstrated that inflammatory and/or metabolic biomarkers are mediators of the inflammatory process of OA[4]. In addition, there is increasing evidence for a potential role of vitamin D deficiency in OA. Vitamin D receptor (VDR) is expressed in chondrocytes, osteoclasts and osteoblasts[5], and vitamin D can reduce bone turnover and cartilage degradation, and thus it has the potential to delay the development and progression of OA[6].

Interestingly, several experimental studies have reported that vitamin D may reduce the inflammatory response by modulating human monocyte function or VDR signalling[7,8]. Observational studies have shown that vitamin D deficiency is associated with increased inflammation in chronic conditions, including asthma, inflammatory bowel disease and rheumatoid arthritis (RA)[9,10]. Such evidence suggests that increased inflammation may be a key underlying mechanism linking vitamin D deficiency to OA, and vitamin D could modify OA disease progression through inhibition of inflammation. Previous studies have examined the effect of vitamin D supplementation on inflammatory biomarkers in older adults

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; hs-CRP, high-sensitivity C-reactive protein; OA, osteoarthritis.

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and patients with some chronic diseases, and have shown inconsistent results[11–14], but no study has reported whether vitamin D supplementation has effects on inflammatory and metabolic biomarkers in OA patients.

Recently we reported that, compared with placebo, vitamin D supplementation had no significant effects on MRI-measured tibial cartilage volume or the Western Ontario and McMaster Universities Arthritis Index (WOMAC) assessed knee pain[15], but significantly reduced MRI-measured joint effusion–synovitis in patients with symptomatic knee OA[16]. This suggests that vitamin D supplementation could have anti-inflammatory effects by regulating serum levels of inflammatory or metabolic biomarkers in knee OA patients. The aim of the current study was, therefore, to determine whether vitamin D supplementation affected serum inflammatory and metabolic biomarkers and whether variation in vitamin D status over 2 years was associated with change in biomarkers in patients with knee OA and vitamin D deficiency.

Methods

Study design and participants

This study was a *post hoc* analysis of the Vitamin D Effect on Osteoarthritis (VIDEO) study, which was a multicentre randomised, double-blind, placebo-controlled trial in knee OA patients with vitamin D deficiency. The method and protocol of the trial were described previously[17]. The trial was conducted from June 2010 to December 2013.

In brief, eligible participants who had knee symptomatic OA (assessed using American College of Rheumatology criteria)[18] at least for 6 months and had pain of >20 mm on a 100-mm visual analogue scale (VAS) with low levels of 25-hydroxyvitamin D (25(OH) D, between 12·5 and 60 nmol/l) were enrolled in Tasmania and Victoria, Australia. Participants with the following conditions were excluded: severe radiographic changes (grade 3 of Altman and Gold Atlas)[19], severe knee pain on standing (>80 mm on a 100-mm VAS), contraindications to MRI, RA or psoriatic arthritis, lupus, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, conditions affecting oral drug absorption, anticipated knee or hip surgery within the next 2 years, history of significant trauma of knees (e.g. arthroscopy or injury to ligaments or menisci within 1 year preceding the study) and history of taking vitamin D or other investigational drugs, such as some compound drugs including vitamin D that affected serum vitamin D levels, within the last 30 d[17].

After the trial had been completed, 200 participants were randomly selected for the measurements of inflammatory and metabolic biomarkers from Tasmania.

Ethics approval was received from Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference no. CF10/1182-2010000616).

Randomisation and treatment

Participants were allocated to either vitamin D or placebo arm at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a centrally automated allocation procedure with security in place to ensure that allocation data cannot be accessed or influenced by any person from the investigative team. Participants received oral vitamin D capsules at a dose of 1·25 mg vitamin D_3 (cholecalciferol) per month for 24 months in the treatment group. Participants received an identical inert placebo capsule in the placebo group[17].

Serum inflammatory and metabolic biomarker level measurement

All fasting blood samples were collected at baseline and at 24 months. The measurements were performed according to the manufacturer's instruction. Serum levels of high-sensitive C-reactive protein (hs-CRP), IL-6, IL-8, IL-10, resistin, leptin, adiponectin, adipsin and apelin-36 were measured. Serum hs-CRP was measured by enzyme immunoassays (IBL Inc.). Serum leptin and apelin-36 were measured by ELISA (Phoenix Pharmaceuticals Inc.). Serum adiponectin, adipsin and resistin were measured by ELISA (Millipore Inc.). Serum IL-6, IL-8 and IL-10 were measured by Bio-plex Luminex assay kits (Bio-Rad Laboratories Inc.). The inter-assay and intra-assay CV were <10 and <15 % for all inflammatory biomarkers.

Serum vitamin D level measurement

Serum 25-hydroxyvitamin D (25(OH)D) was measured at baseline, and at months 3 and 24 using direct competitive chemiluminescent immunoassays (DiaSorin Inc.). The intra-assay and inter-assay CV were 3·2 and 6·0 %, respectively[15]. In addition, the season of blood sample was recorded. In this study, we defined serum 25-(OH)D below than 50 nmol/l as vitamin D deficiency.

Variation in vitamin D status

Participants were classified into two groups according to the levels of 25(OH)D at months 3 and 24 as follows: not consistently sufficient (serum 25(OH)D ≤ 50 nmol/l at either month 3 or 24), and consistently sufficient (serum 25(OH)D > 50 nmol/l at both months 3 and 24).

Assessment of effusion–synovitis and cartilage volume

MRI scans of the study knee were obtained according to a standardised protocol using a 1·5 T whole-body MRI unit with a commercial transmit-receive extremity coil at baseline and 2 years.

Effusion–synovitis was assessed using T2-weighted fast-spin echo sequences at four regions (suprapatellar pouch, central portion, posterior femoral recess and subpoploteal recess). Effusion–synovitis in each subregion was scored individually according to Whole-Organ Magnetic Resonance Imaging Score, grading collectively from 0 to 3 in terms of the estimated maximal distention of the synovial cavity: 0 refers to normal; 1 to <33 % of maximum potential distention; 2 to 33–66 % of maximum potential distention; and 3 to >66 % of maximum

potential distention. The presence of effusion–synovitis of the whole joint was defined as a score of ≥2 in any subregion[16].

Effusion–synovitis volumes at 4 regions were isolated from the total volume selecting each region of interest according to the intra-articular fluid-equivalent signal on a section-by-section basis and then resampled by means of bilinear and cubic interpolation for final 3D rendering using OsiriX imaging software (32-bit version 5.9; Pixmeo SARL). The intra-class correlation coefficients were from 0·96 to 0·97[20].

Cartilage volume was determined using the previously described image processing techniques[17]. The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis and then resampled using bilinear and cubic interpolation for final three-dimensional rendering using OsiriX imaging software. The CV was 2·1 to 2·2 %[20]. Total tibial cartilage volume was calculated as the sum of the medial tibia and lateral tibial cartilage plates.

Change in cartilage volume and effusion–synovitis volume was calculated as follows: Absolute change (ml) = (follow-up volume) − (baseline volume).

Anthropometrics

Height was measured to the nearest 0·1 cm (with shoes removed) using a stadiometer (Leicester Height Measure; Invicta Plastics Ltd). Weight was measured to the nearest 0·1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307; Heine). BMI (kg/m^2) was calculated.

Data analyses

Very few studies have examined the effect of vitamin D supplementation on inflammatory biomarkers; therefore, there was limited information to inform our sample size calculation. On the basis of a systematic review, the absolute difference in serum hs-CRP between people with OA and healthy controls is estimated as 1·19 mg/l[21]. Data from the Tasmanian Older Adult Cohort Study provided a SD estimate of 2·78 mg/l. With these estimates, a sample size of eighty-seven in each group would give 80 % power with a 5 % probability of type 1 error ($\alpha = 0{\cdot}05$, $\beta = 0{\cdot}8$)[22] to detect this effect size for hs-CRP.

Baseline characteristic differences between the vitamin D supplementation and placebo groups were compared using Student's t tests or χ^2 tests as appropriate. Box–Cox transformation was used, when variables were not normally distributed, and transformed variables were used in the following analyses. The differences in changes of inflammatory biomarkers between treatment and placebo groups were analysed using linear mixed effects model with adjustment for age, sex, BMI and seasonal change of blood sampling. The within-subject correlation between the repeated measures, including baseline and follow-up data, was taken into account using the individual participant identification as a random effect. The effect of vitamin D supplementation on biomarkers was evaluated by the interaction between treatment and time (i.e. month). The differences in changes of inflammatory biomarkers between not consistently sufficient and consistently sufficient groups were analysed using linear mixed effects model with adjustment for age, sex, BMI and seasonal change of blood sampling. Subgroup analyses were performed in participants with or without effusion–synovitis at baseline. We also have taken the weight change as confounder into analyses to make sure that the weight change did not play a role in this study. Further adjustment for changes in cartilage volume and effusion–synovitis volume was performed to account for the effect of disease progression on serum biomarker change. We used Stata 12.0 for Windows (StataCorp LP) for all analyses. A P value <0·05 (two-tailed) was regarded as statistically significant.

Results

Baseline characteristics of participants

A total of 599 participants were screened for eligibility, 413 participants were enrolled and randomly assigned to vitamin D or placebo group (261 participants in Hobart and 152 participants in Melbourne) and 340 participants (82·0 % retention rate in Hobart and 83·6 % retention rate in Melbourne) completed the study (Fig. 1). A total of 200 participants from Hobart were randomly selected for the inflammatory biomarker measurements: ninety-four participants from the placebo group and 106 from the vitamin D treatment group. The mean age of participants was 63·1 years: 107 (53·5 %) were women and mean BMI was 29·5 kg/m^2. There were no significant differences in baseline characteristics (age, sex, BMI, serum 25(OH)D level, serum inflammatory biomarkers levels and season of blood sample) between the placebo and vitamin D groups (Table 1). There were no significant differences in baseline characteristics between those included and not included in this study (data not shown). Significant differences in baseline characteristics, inflammatory and metabolic biomarkers between consistently sufficient and not consistently sufficient groups were not found (data not shown).

Vitamin D supplementation and inflammatory and metabolic biomarkers

The mean serum 25(OH)D level increased significantly in the vitamin D treatment group (44·9 nmol/l) compared with the placebo group (7·0 nmol/l) over 2 years. The effect of vitamin D treatment on cytokines and adipokines is shown in Table 2. Vitamin D supplementation had no significant effect on change in serum inflammatory and metabolic biomarkers. There were no statistically significant differences in changes in any biomarkers between the placebo and vitamin D groups. After further adjustment for potential confounders including the seasonal change of blood sample, the differences between groups remained non-significant in the mixed effect model (Table 2). Within the group, serum resistin increased by 1·9 pg/ml (95 % CI 0·1, 3·8) in the placebo group and by 4·4 pg/ml (95 % CI 2·7, 6·2) in the vitamin D group from baseline to month 24. Difference in change in serum resistin between two groups was of borderline statistical significance ($P = 0{\cdot}05$). Serum adipsin increased by 0·2 ng/ml (95 % CI 0·1, 0·4) in the vitamin D group, but did not increase in the

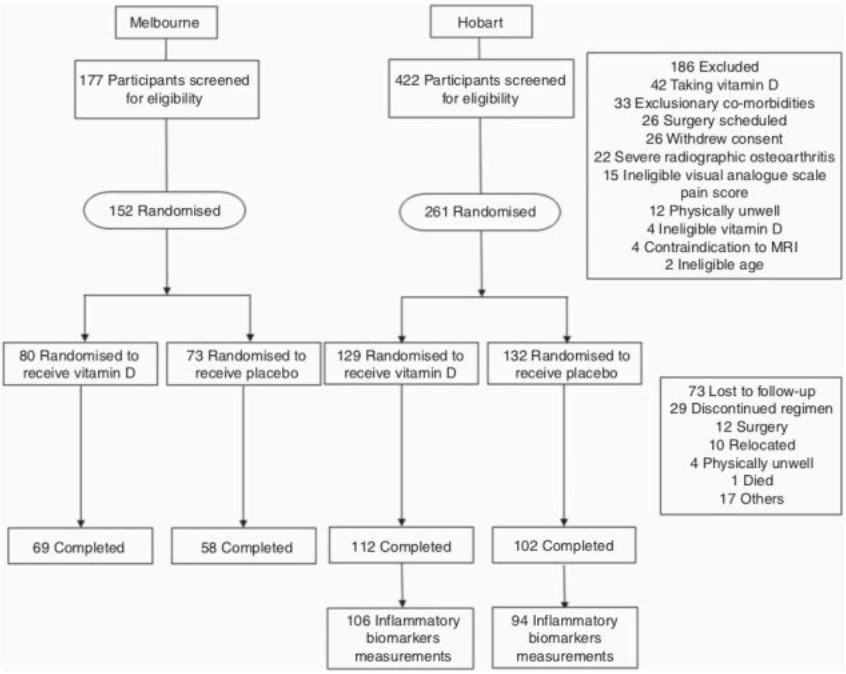

Fig. 1. Flowchart of the study.

placebo group from baseline to month 24. Serum hs-CRP, IL-6, IL-8, IL-10, leptin, adiponectin and apelin did not change significantly over 24 months in either group.

Further analyses were performed in participants who had baseline effusion–synovitis or not. Results remained largely unchanged (data not shown). The results remained unchanged after further adjustment for change in weight, and changes in cartilage volume and effusion–synovitis volume (data not shown).

Vitamin D status and change in biomarkers

There were no statistically significant differences in changes of these biomarkers between consistently sufficient and not consistently sufficient groups (Table 3). In the consistently vitamin D sufficient group, there were significant increases in serum resistin and adipsin (3·8 pg/ml and 0·3 ng/ml, respectively) and a significant decrease in serum IL-8 (3·0 pg/ml) from baseline to month 24 (Table 3). In contrast, there was no significant change over the study period in the not consistently sufficient group. Serum IL-6, IL-10, CRP, leptin, adiponectin and apelin did not change significantly from baseline to month 24 in either group.

The results remained largely unchanged if patients with and without baseline effusion–synovitis were separated for analyses (data not shown). Results remained largely unchanged after further adjustment for changes in weight, cartilage volume and effusion–synovitis volume (data not shown). In addition, no significant associations were found between the changes in inflammatory/metabolic biomarkers and change in 25(OH)D over 24 months, and the results remained largely unchanged after further adjustment for the baseline serum 25(OH)D level (except for change in resistin, $P=0·04$) (data not shown).

Discussion

To the best of our knowledge, this study is the first to explore the effect of vitamin D supplementation on inflammatory and metabolic biomarkers in patients with knee OA and to compare the effect of vitamin D sufficiency on levels of serum inflammatory and metabolic biomarkers in OA patients. Vitamin D supplementation had no significant effects on serum inflammatory and metabolic biomarkers in patients with knee OA. Furthermore, there were no significant differences in serum inflammatory and metabolic biomarkers between those who were consistently sufficient and those who were not over 24 months. These results suggest that vitamin D supplementation and maintaining sufficient vitamin D status may not have effects on systemic inflammation in knee OA patients.

Low-grade systemic inflammation triggered by abnormally inflammatory or metabolic biomarkers has been implicated in the OA pathogenesis. There is a vast amount of evidence linking inflammatory biomarkers and OA, as well as the

association between vitamin D and inflammation. Serum hs-CRP levels were statistically significantly higher in the OA group than in the control group and were associated with increased pain and decreased physical function[21]. Serum IL-6 was correlated with radiographic OA, knee cartilage loss and increased knee pain over time[23,24]. Adipokines such as leptin and resistin may disrupt cartilage homoeostasis by directly inducing joint structural degradation or regulating local inflammatory processes and are regarded as metabolic biomarkers in the inflammatory process of OA[25,26]. Serum leptin was associated with reduced knee cartilage volume and increased loss of cartilage thickness[27]. Furthermore, high serum leptin and IL-6 were associated with reduced 25(OH)D levels over time[28]. These suggest that systemic inflammation triggered by inflammatory or metabolic biomarkers may be a key underlying mechanism linking vitamin D deficiency to OA.

Although there has been no previous RCT examining the effect of vitamin D supplementation on inflammatory and metabolic biomarkers in knee OA patients, some RCT have examined the effect in healthy individuals, older adults or patients with other chronic diseases such as obesity, asthma, diabetes and chronic kidney disease, and reported inconsistent results[29]. The inconsistency between these study findings may be caused by small sample sizes, diverse characteristics of participants, treatment with different doses and measurements of different inflammatory and metabolic biomarkers for the different clinical trials[30]. For example, two RCT with small sample size were conducted in patients with diabetes. One study reported that supplementation with 1·25 mg vitamin D per 2 weeks for 12 weeks in 60 patients significantly reduced serum hs-CRP level compared with the placebo group[31], but another study reported that treatment with 1·25 mg/week vitamin D and/or 1000 mg Ca/d twice for 8 weeks did not result in significant difference in change in serum CRP and leptin, but did result in significant reduction in serum IL-6 and TNF-α compared with the placebo group in 118 diabetic patients[12].

Our results are consistent with findings from RCT in healthy or older adults without specific health conditions as vitamin D supplementation in these groups has shown no effect on inflammatory and metabolic biomarkers[32–35]. A randomised placebo-controlled trial was conducted in elderly adults without a specific disease in Australia, aiming to examine the effects of vitamin D supplementation for 12 months on hs-CRP, leptin, adiponectin, IL-6 and IL-10[36]. IL-6 was numerically higher in those participants supplemented with 1500 µg/month vitamin D compared with those supplemented with 750 µg/month,

Table 1. Baseline characteristics of participants
(Mean values and standard deviations; numbers and percentages; medians and interquartile ranges (IQR))

	Vitamin D group (*n* 106)		Placebo group (*n* 94)		
	Mean	SD	Mean	SD	*P**
Age (years)	63·3	7·5	62·8	7·3	0·60
Women					0·29
n	53		54		
%	50·0		57·4		
BMI (kg/m²)†	29·4	7·5	29·6	4·0	0·80
Serum 25(OH)D (nmol/l)	42·5	11·7	43·5	12·6	0·57
Serum biomarker					
hs-CRP (µg/ml)‡					0·62
Median	1·5		1·3		
IQR	0·8, 2·6		0·7, 2·5		
IL-6 (pg/ml)‡					0·81
Median	1·4		1·2		
IQR	0·4, 3·8		0·4, 3·7		
IL-8 (pg/ml)‡					0·98
Median	7·8		7·6		
IQR	5·7, 10·4		6·1, 10·9		
IL-10 (pg/ml)‡					0·56
Median	0·9		0·6		
IQR	0·3, 5·2		0·3, 3·5		
Resistin (pg/ml)	38·4	14·9	39·3	13·2	0·32
Leptin (ng/ml)‡					0·90
Median	19·2		23·6		
IQR	9·4, 58·1		9·7, 44·1		
Adiponectin (ng/ml)‡					0·11
Median	32·9		26·5		
IQR	18·2, 50·3		15·5, 43·8		
Adipsin (ng/ml)	4·0	1·5	3·9	1·2	0·84
Apelin (ng/ml)	1·0	0·3	1·0	0·4	0·84

25(OH)D, 25-hydroxyvitamin D; hs-CRP, high-sensitivity C-reactive protein.
* Student's *t* tests or χ^2 tests.
† BMI was calculated as weight in kg divided by height in m².
‡ Skewed distribution.

Table 2. Comparisons of change in inflammatory biomarkers between vitamin D and placebo groups over 24 months
(Mean values and 95 % confidence intervals)

	Vitamin D group (change, *n* 106)		Placebo group (change, *n* 94)		Between-group (difference)		
	Mean*	95 % CI	Mean*	95 % CI	Mean†	95 % CI	*P*
Serum 25(OH)D (nmol/l)	45·0	40·8, 49·0	7·4	3·0, 11·8	37·5	31·5, 43·6	0·00
hs-CRP (µg/ml)	0·3	−0·2, 0·7	−0·0	−0·5, 0·5	0·3	−0·4, 1·0	0·43
IL-6 (pg/ml)	−2·3	−5·6, 0·9	−0·7	−4·2, 2·8	−1·6	−6·4, 3·2	0·51
IL-8 (pg/ml)	−3·1	−6·5, 0·3	−0·0	−3·7, 3·6	−3·1	−8·1, 2·0	0·24
IL-10 (pg/ml)	−2·0	−9·3, 5·3	−0·7	−8·5, 7·1	−1·3	−12·0, 9·4	0·81
Resistin (pg/ml)	4·4	2·7, 6·2	1·9	0·1, 3·8	2·5	−0·0, 5·1	0·05
Leptin (ng/ml)	−0·2	−3·4, 3·0	−0·8	−4·2, 2·6	0·6	−4·0, 5·3	0·79
Adiponectin (ng/ml)‡	0·01	−0·02, 0·04	0·00	−0·03, 0·03	0·01	−0·04, 0·06	0·66
Adipsin (ng/ml)	0·2	0·1, 0·4	0·1	−0·0, 0·3	0·1	−0·1, 0·3	0·39
Apelin (ng/ml)	−0·1	−0·1, 0·0	0·0	−0·1, 0·1	−0·1	−0·2, 0·0	0·13

25(OH)D, 25-hydroxyvitamin D; hs-CRP, high-sensitivity C-reactive protein.
* Changes in inflammatory biomarkers are generated from mixed models adjusted for age, sex, BMI and change in season of blood sampling.
† Between-group difference was calculated using vitamin D group values minus placebo group values.
‡ Box–Cox transformation.

Table 3. Comparison of change in inflammatory biomarkers between different vitamin D status over 24 months (Mean values and 95 % confidence intervals)

	Consistently sufficient (change, *n* 139)		Not consistently sufficient (change, *n* 61)		Between-group (difference)		
	Mean*	95 % CI	Mean*	95 % CI	Mean	95 % CI†	*P*
Serum 25(OH)D (nmol/l)	37·8	34·1, 41·6	3·1	−2·6, 8·7	34·8	28·0, 41·6	0·00
hs-CRP (μg/ml)	0·2	−0·2, 0·7	−0·1	−0·7, 0·6	0·3	−0·5, 1·1	0·46
IL-6 (pg/ml)	−2·8	−5·7, 0·0	1·3	−3·0, 5·6	−4·1	−9·3, 1·0	0·12
IL-8 (pg/ml)	−3·0	−6·0, −0·0	1·4	−3·1, 6·0	−4·5	−9·9, 1·0	0·11
IL-10 (pg/ml)	−3·3	−9·6, 3·1	2·9	−6·8, 12·6	−6·2	17·8, 5·4	0·29
Resistin (pg/ml)	3·8	2·3, 5·4	1·9	−0·4, 4·3	1·9	−0·9, 4·7	0·18
Leptin (ng/ml)	−0·1	−2·9, 2·7	−1·3	−5·5, 2·9	1·1	−3·9, 6·2	0·66
Adiponectin (ng/ml)‡	0·02	−0·01, 0·04	−0·01	−0·06, 0·03	0·03	−0·02, 0·08	0·26
Adipsin (ng/ml)	0·3	0·1, 0·4	0·1	−0·1, 0·3	0·2	−0·1, 0·4	0·13
Apelin (ng/ml)	−0·0	−0·1, 0·0	−0·1	−0·1, 0·0	0·0	−0·1, 0·1	0·72

25(OH)D, 25-hydroxyvitamin D; hs-CRP, high-sensitivity C-reactive protein.
* Changes in inflammatory biomarkers are generated from mixed models adjusted for vitamin D treatment, age, sex, BMI and change in season of blood sampling.
† Between-group difference was calculated using consistently sufficient group values minus not consistently sufficient group values.
‡ Box–Cox transformation.

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although this was not significant. In our current study, we found that vitamin D supplementation had no significant effect on serum inflammatory and metabolic biomarkers, with the exception of a possible effect on serum resistin, in patients with knee OA. Serum resistin increased twofold in the vitamin D group than in the placebo group, but the difference was of borderline significance. Although evidence shows that serum resistin is a pro-inflammatory cytokine and is positively associated with severity of OA[37], the clinical relevance of this finding is unknown. Subgroup analyses were performed in participants who had baseline effusion–synovitis or not, and results remained unchanged. We previously reported that vitamin D supplementation relieved the progression of effusion–synovitis in patients with an inflammatory OA phenotype[16]. These indicated that vitamin D supplementation would have effects on local rather than systemic inflammation. The underlying mechanisms are unclear and need to be explored by further studies.

A high proportion of participants in the placebo group achieved sufficient vitamin D level in month 24 (62 % participants >50 nmol/l) in the VIDEO study, which may have masked the effect of vitamin D supplementation on inflammatory and metabolic biomarkers. Therefore, we performed further *post hoc* analyses to examine whether maintaining sufficient vitamin D level over the treatment period had effects on the biomarkers. Although serum resistin and adipsin increased, and serum IL-8 decreased from baseline to month 24 in the consistently vitamin D sufficient group, changes in serum resistin, adipsin and IL-8 were not significantly different between the patients with different vitamin D status. Until now, only one cross-sectional study compared the level of inflammatory cytokines (IL-1β, 2, 4, 5, 6, 8, 10, 12, 13 and hs-CRP) in different serum vitamin D status (deficient, insufficient or sufficient) in patients with knee OA[38], and did not find that vitamin D status was associated with circulating inflammatory biomarkers. These results were generally consistent with the findings in our current study. Maintaining serum vitamin D sufficiency may not have effects on systemic inflammation in knee OA patients.

We used the direct competitive chemiluminescent immunoassays (Diasorin Liaison assay) to measure the serum 25(OH)D, which may not be as sensitive and specific as HPLC methodology; however, it showed good correlation with HPLC, and would also be specific for both 25(OH)D_2 and 25(OH)D_3[39,40]. The vitamin D status, measured via serum 25(OH)D concentrations, can be affected by factors such as obesity. In addition, expression of vitamin D-dependent genes could be served as a marker of vitamin D status, and this may also influence the effect of vitamin D supplementation on inflammatory markers[41,42]. These need to be explored in future studies.

There were some limitations of the current study. First, it was a *post hoc* analysis within a subsample of an RCT, which was not designed to examine the effect of vitamin D supplementation on inflammatory and metabolic biomarkers in patients with knee OA. The findings need to be confirmed by further RCT using these biomarkers as the primary end points. Second, this study had reduced sample size than what was designed for the original study. However, the sample size is sufficient to detect a significant difference for hs-CRP. Third, there was a high proportion of participants in the placebo group who achieved sufficient vitamin D levels at months 3 and 24 (62 % participants >50 nmol/l) in the RCT study, which could have diluted the effect of vitamin D supplementation. Thus, we performed further *post hoc* analyses using variations in vitamin D status over the treatment period and the results were consistent. Fourth, three time points (baseline, 3 months and 24 months) may not be adequate to define the 'consistently or inconsistently vitamin D sufficient', and the inflammatory and metabolic markers were not measured at an intermediate time point. It was unknown whether vitamin D supplementation had intermediate effects on inflammatory markers in knee OA patients. Therefore, further studies are required.

Conclusion

Vitamin D supplementation and maintaining vitamin D sufficiency did not alter serum levels of inflammatory and metabolic

biomarkers over 2 years in knee OA patients who were vitamin D insufficient, suggesting that they may not affect systemic inflammation in knee OA patients.

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All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. S. Z. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. S. Z. and C. D. designed the study, collected data, carried out data analyses, interpreted the results and drafted the manuscript. B. W., W. H., Z. Z., X. W., X. J., B. A., F. C., A. W., T. W., D. A., L. B. and G. J. were involved in collecting the data, helping the data analyses, interpreting the results and revising the manuscript.

The authors declare that there are no conflicts of interest.

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Effect of vitamin D on depressive symptoms

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Original Study

Effect of Vitamin D Supplementation on Depressive Symptoms in Patients With Knee Osteoarthritis

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A B S T R A C T

Objectives: To determine the effect of vitamin D supplementation and maintaining sufficient serum vitamin D on depressive symptoms in patients with knee osteoarthritis (OA) and vitamin D deficiency.
Design: A prespecified secondary analysis of a multicentre, randomized, double-blind, placebo-controlled trial. Participants were randomly assigned to receive oral vitamin D_3 (50,000 IU, n = 209) or placebo (n = 204) monthly for 24 months. In addition, participants who completed the trial were classified into 2 groups according to their serum 25(OH)D levels at month 3 and 24 as follows: not consistently sufficient (serum 25(OH)D ≤ 50 nmol/L at month 3 and/or 24), and consistently sufficient (serum 25(OH)D > 50 nmol/L at both month 3 and 24). Multilevel mixed-effect models were used to compare differences of change in PHQ-9 scores between groups.
Setting and Participants: This clinical trial was conducted in participants with symptomatic knee OA and vitamin D deficiency from June 2010 to December 2013 in Tasmania and Victoria, Australia.
Measures: The primary outcome was the depressive symptoms change over 24 months, which was measured using the Patient Health Questionnaire (PHQ-9, 0-27).
Results: Of 599 participants who were screened for eligibility, 413 participants were enrolled (mean age: 63.2 years; 50.3% female) and 340 participants (intervention n = 181, placebo n = 159, 82.3% retention rate) completed the study. The baseline prevalence of depression (PHQ-9 score ≥5) was 25.4%. Depressive symptoms improved more in the vitamin D supplementation group compared to the placebo group [β: −0.66, 95% confidence interval (CI): −1.22 to −0.11, *P* for difference = .02] and in the participants who maintained vitamin D sufficiency compared to those who did not (β: −0.73, 95% CI: −1.41 to −0.05, *P* for difference = .04) over 24 months.
Conclusions/Implications: These findings suggest that vitamin D supplementation and maintaining adequate vitamin D levels over 24 months may be beneficial for depressive symptoms in patients with knee OA.

Depression is a major global public-health problem and is projected to be the second leading cause of disease burden by the year 2030.[1] Because of population aging, depression in later life is a common comorbidity of many chronic diseases.[2] Although depression is less prevalent among older than younger adults, it still has detrimental consequences.[3] Similarly, osteoarthritis (OA) is the most

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prevalent chronic joint disease and the leading cause of disability in individuals and has a substantial financial burden on the health system.[4,5] It is predicted that 130 million individuals who are older than 60 years will suffer from OA worldwide by 2050.[6] Depression and depressive symptoms are common among individuals with OA. Stubbs and colleagues reported that 19.9% of those with OA had depressive symptoms, with a relative risk of 1.17 in those with OA compared to those without.[7] Concomitant depression in OA patients contributes to its increased disease burden and troubles with disease management.[8,9]

Vitamin D deficiency or insufficiency is a global public health problem and affects nearly one billion people worldwide.[10] Vitamin D deficiency is associated with a range of mental disorders including depression.[10] Vitamin D receptors exist in the brain, and vitamin D can boost the levels of brain chemical monoamines, which is necessary for a positive mood and has possible neuroprotective roles that vitamin D may play through its effects on inflammation.[11–14] Furthermore, a positive association between vitamin D deficiency and depression has been reported in previous epidemiologic and clinical studies in the general population, including children, older adults, and patients with other chronic diseases[15–17]; however, it remains unknown whether the relationship is causal.[15] Although the biological mechanisms underlying the associations between vitamin D deficiency and depression are unclear and may be complicated, the therapeutic potential of vitamin D supplementation for depression has been highlighted.[16] Thus far, randomized controlled trial (RCT) evidence examining whether vitamin D supplementation can improve depression has been investigated in the general population and shows inconsistent findings. The contradictory evidence may be caused by variations in baseline vitamin D levels, vitamin D dosages, sample sizes, duration of follow-up, outcome measurements, and investigated populations.[18] A meta-analysis reviewed 9 clinical trials and reported no significant reduction in depression after vitamin D supplementation, but most of the studies focused on individuals with low levels of depression or who had sufficient vitamin D at baseline.[19] Further well-designed vitamin D supplementation RCTs among individuals who are both depressed and vitamin D deficient are needed.

Although depression is prevalent in OA patients and a positive association between vitamin D deficiency and depression has been demonstrated, no study has examined the effect of vitamin D supplementation on depressive symptoms in people with OA so far. This study primarily aims to determine the effect of vitamin D supplementation on depressive symptoms in patients with knee OA and vitamin D deficiency. An ancillary aim of this study is to determine whether maintaining sufficient serum vitamin D has beneficial effects on depressive symptoms.[20]

Methods

Trial Design and Participants

This study is a prespecified secondary analysis of the Vitamin D Effect on Osteoarthritis (VIDEO) study, which was conducted from June 2010 to December 2013. VIDEO was a multicenter, randomized, double-blind, placebo-controlled trial to determine the effect of vitamin D supplementation on knee structures and symptoms among patients with symptomatic knee OA. This secondary analysis aims to determine the effect of vitamin D supplementation on depressive symptoms in patients with knee OA and vitamin D deficiency. The ancillary aim of determining whether maintaining sufficient serum vitamin D has beneficial effects on depressive symptoms was added because there was a high proportion of participants having unforeseen improvements in serum 25-hydroxyvitamin D (25[OH]D) levels in the placebo group of our RCT as previously published.[21]

The methods were described in the published protocol of the trial,[22] and the results for primary outcomes have been published.[20] Participants who had symptomatic knee OA, which was assessed using the American College of Rheumatology criteria,[23] for at least 6 months with knee pain >20 mm on a 100-mm visual analog scale and serum levels of 25-hydroxyvitamin D (25[OH]D) between 12.5 and 60 nmol/L were enrolled in Tasmania and Victoria, Australia. Participants with grade 3 radiographic changes (Altman and Gold Atlas), severe knee pain on standing (>80 mm on a 100-mm visual analog scale), other rheumatic diseases such as rheumatoid arthritis, psoriatic arthritis and lupus, contraindication to magnetic resonance imaging, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, anticipated knee or hip surgery within the next 2 years, and history of taking vitamin D within the previous 1 month were excluded.[22]

All participants provided informed written consent and the study was approved by the Ethics Committee in Tasmania and Melbourne (reference number: H1040 and CF10/1182-2010000616, respectively).

Randomization and Intervention

Participants were allocated to either the vitamin D or placebo arm at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a centrally automated allocation procedure with security in place to ensure allocation data could not be accessed or influenced by any person from the investigative team. Participants, research coordinators, and investigators were all blinded to treatment assignment. Blinding was maintained until all data were collected, cleaned, confirmed for accuracy, and analyses of primary outcomes were performed.

Participants received 50,000 IU (1.25 mg) oral vitamin D_3 capsule (cholecalciferol) monthly for 24 months or an identical inert placebo.[22] Both the vitamin D_3 compound and placebo were prepared by and purchased from Nationwide Compounding Pharmacy, Melbourne, Australia.

Patient Health Questionnaire–9

Depressive symptoms were assessed using the Patient Health Questionnaire–9 (PHQ-9) at baseline and months 3, 6, 12, and 24. PHQ-9 is a valid and reliable self-reported depression instrument, which is widely used in multipurpose diagnoses, severity measures in the clinic, as well as assessing depression outcomes in research.[24,25] It is a 9-item questionnaire with a score range of 0 to 27, with each item being scored from 0 to 3 (not at all, several days, more than half days, and nearly every day). Using the mental health professional interview as the criterion standard, PHQ-9 scores of 5 to 9, 10 to 14, 15 to 20, and >20 represent mild, moderate, moderately severe, and severe depression, respectively. A PHQ-9 score ≥10 has a sensitivity of 88% and a specificity of 88% for major depression.[24]

Serum 25(OH)D Measurement

Serum 25(OH)D was measured at baseline and month 3 and 24 using direct competitive chemiluminescent immunoassays, which is an accurate and reproducible automated tool[26] (DiaSorin Inc, Macquarie Park, New South Wales, Australia). The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%, respectively.[20] The season of blood sample was recorded.

Knee Symptoms Measurement

Knee symptoms were assessed at baseline and months 3, 6, 12, and 24 using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), which is a widely used instrument to evaluate

the functional capacity in patients with OA and demonstrates high performance in clinical trials.[27] The Index contains 24 questions (5 related to pain, 2 to stiffness, and 17 to physical function), with scores ranging from 0 (none) to 100 (severe) for each question. The total WOMAC score (0–2400) is the sum of subscale scores including pain (0–500), stiffness (0–200), and physical function (0–1700).

Physical Activity

Physical activity (PA) was assessed by the International Physical Activity Questionnaire, which has been developed and tested for use in adults (age range of 16–69 years). PA status (insufficiently active, sufficiently active, and highly active) was calculated according to the scoring protocol available at http://www.ipaq.ik.se. The International Physical Activity Questionnaire was valid and reliable for self-reports and monitoring population levels of physical activity among adults (18-65 years old) in diverse settings.[28]

Anthropometrics and Social Demographic Characteristics Data

Height was measured to the nearest 0.1 cm (with shoes removed) using a stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Leicester, United Kingdom). Weight was measured to the nearest 0.1 kg (with shoes and bulky clothing removed) using electronic scales (Heine S-7307; Heine, NH). Body mass index (BMI) was calculated.[22] Obesity status were defined as the normal weight, overweight, and obese depending the BMI according the criteria.[29] Patients filled out a questionnaire that collected information on education history (grade 0: less than high school, grade 1: high school degree, and grade 2: superior than high school degree), current regular smoker (yes or no), current medical conditions, and medication uses.

Statistical Methods

The primary analysis compared the effect of vitamin D supplementation on depressive symptoms as measured by the PHQ-9 between the vitamin D group and the placebo group. Given the previously published high level of correction of deficiency in the placebo group (62% reached vitamin D sufficiency at month 24 in the placebo group), a secondary analysis was also performed by vitamin D status.[20,21] Participants were classified into 2 groups according to their serum 25(OH)D levels at month 3 and 24 as follows: not consistently sufficient (serum 25[OH]D $\leq$ 50 nmol/L at month 3 and/or 24) and consistently sufficient (serum 25[OH]D $>$ 50 nmol/L at both month 3 and 24).

Baselined on Lowe's study,[25] we anticipated a standard deviation of 5.4 for the change in PHQ-9. The sample size calculation assumed $\alpha = 0.05$ and $\beta = 0.20$ and was performed based on the Cohen formula.[30] With 400 participants, a difference between groups of 1.2 units on the score is detectable with 80% power.

Differences in baseline characteristics between vitamin D and placebo groups were compared using independent t tests or chi-square tests as appropriate. Repeated measures mixed-effect models were used to examine changes in PHQ-9 scores over 24 months in the vitamin D supplementation versus the placebo group and the group that maintained vitamin D sufficiency between month 3 and 24 versus the group that did not maintain vitamin D sufficiency. The models were adjusted for age, sex, and BMI. Further adjustments for knee pain, joint function, baseline PHQ-9 score, serum 25(OH)D level, self-reported depression, and/or antidepressant medication were performed. Missing data due to loss to follow-up or nonresponses were accounted for in the multilevel mixed-effect model. Subgroup analysis was performed to examine whether the effect of vitamin D supplementation and maintaining vitamin D sufficiency varied by the presence of depressive symptoms at baseline. A reduction of PHQ-9 score of greater than 2.59/27 was used as the cut-off for the minimal clinically important difference (MCID) based on the Lowe recommendation, which was developed for those with major depression.[25]

All tests were 2-sided and a P value $< .05$ was considered as statistically significant. Stata version 12.0 was used to perform statistical analyses.

Results

Baseline Characteristics of Participants

Figure 1 shows the flow of study participants. Five hundred ninety-nine participants were screened for eligibility, and 413 participants were enrolled (Figure 1). Two hundred nine and 204 participants were randomly assigned to receive vitamin D and placebo, respectively. Over 24 months, 340 participants (intervention n = 181, placebo n = 159, 82.3% retention rate) completed the study. The mean age of the participants was 63.2 (standard deviation 7.0) years; 208 (50.3%) were women, and the mean BMI was 29.6 (standard deviation 5.0). The baseline prevalence of depression (PHQ-9 score $\geq$5) was 25.4% (mild and moderate to severe depression was 17.6% and 7.8%, respectively). The actual range of PHQ-9 score in this study was 0 to 24 in the vitamin D supplementation group and 0 to 22 in the placebo group.

Table 1 presents the characteristics of participants stratified by treatment groups. The 2 groups had similar demographic and social-demographic features, medication uses, physical activity, knee symptoms, and prevalence of depression. There were also no significant differences in baseline characteristics, physical activity, knee symptoms, and prevalence of depression between the consistently sufficient and not consistently sufficient groups (data not shown); however, the baseline serum 25(OH)D level and PHQ-9 score was higher in the consistently sufficient group (45.1 vs 41.5, $P = .01$; 3.3 vs 2.4, $P = .046$). The characteristics of participants stratified by maintaining and not maintaining vitamin D sufficiency groups are presented in the Supplementary Table.

Vitamin D Supplementation, Vitamin D Status, and Change in Depressive Symptoms

Whole sample

After 24 months, serum 25(OH)D levels increased from 43.7 $\pm$ 11.8 nmol/L to 84.5 $\pm$ 17.3 nmol/L in the vitamin D group and increased from 43.8 $\pm$ 12.7 nmol/L to 50.6 $\pm$ 17.5 nmol/L in the placebo group, as described previously.[20]

Tables 2 and 3 describe the changes in PHQ-9 scores in vitamin D treatment groups and vitamin D status groups. PHQ-9 scores improved more in the vitamin D supplementation group compared to the placebo group [β: −0.66, 95% confidence interval (CI): −1.22 to −0.11; Figure 2] with adjustment for age, sex, and BMI. The results remained significant with further adjustment for baseline PHQ-9 score, self-reported depression, and usages of antidepressant medication (β: −0.59, 95% CI: −1.15 to −0.04). Although the rate of obesity in the placebo group was higher than the vitamin D group, the results remained largely unchanged after adjustment for obesity (data not shown). PHQ-9 scores also improved more in those participants who maintained vitamin D sufficiency between months 3 and 24 compared to those who did not maintain sufficiency (β: −0.77, 95% CI: −1.45 to −0.08; Table 3 and Figure 3) with adjustment for potential confounders, age, sex, BMI, serum 25(OH)D level, PHQ-9 score, self-reported depression, and use of antidepressant medication at baseline.

The differences between the vitamin D supplementation and placebo groups became smaller but remained significant after further adjustment for WOMAC pain (β: −0.61, 95% CI: −1.17 to −0.06) and

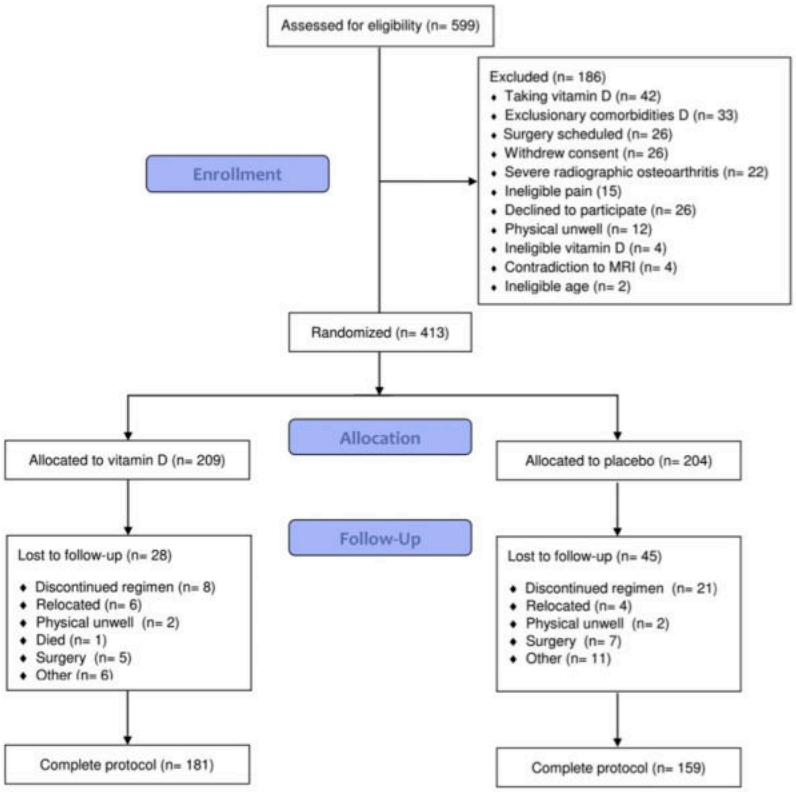

Fig. 1. Flowchart of the study.

WOMAC function (β: −0.59, 95% CI: −1.15 to −0.02). The differences between the vitamin D status groups also diminished but remained significant after further adjustment for WOMAC pain (β: −0.64, 95% CI: −1.24 to −0.03) but became nonsignificant after further adjustment for WOMAC function (β: −0.59, 95% CI: −1.20 to 0.03).

Subgroup analysis by the presence of depression at baseline

In participants with depression (PHQ-9 score ≥5) at baseline, PHQ-9 scores improved significantly in the vitamin D supplementation group (n = 56), placebo group (n = 45), and the group that maintained sufficiency between month 3 and 24 (n = 61) (Tables 2 and 3). There was no significant change in PHQ-9 scores in the group that did not maintain sufficiency (n = 18). Although the differences between the groups were not statistically significant, PHQ-9 scores improved to a greater degree in the vitamin D supplementation group (β: −0.86, 95% CI: −2.64 to 0.91) and the group that maintained vitamin D sufficiency (β: −1.93, 95% CI: −4.14 to 0.28) between month 3 and 24 months compared to the placebo group and participants who did not maintain vitamin D deficiency, respectively. PHQ-9 scores worsened in all subgroups who did not suffer depression at baseline (Tables 2 and 3). Although there was no significant difference between the groups, the worsening was smaller in the vitamin D supplementation group and the group that maintained sufficient vitamin D levels between month 3 and 24.

In the participants with moderate to severe depression at baseline, the proportion who achieved MCID improvement (defined as >2.59/27) in PHQ-9 was 76.9% in the vitamin D supplementation group and 77.8% in the placebo group ($P = .86$).

Discussion

This current study, to our knowledge, is the first to investigate the effect of vitamin D supplementation and maintaining vitamin D sufficiency on depressive symptoms over 24 months in patients with knee OA and vitamin D deficiency. In our sample, the prevalence of depression in knee OA patients was 25.4%. PHQ-9 scores improved in the vitamin D treatment group and the group that maintained vitamin D sufficiency between month 3 and 24, compared to the placebo group and the group that did not maintain sufficient vitamin D, respectively. Clinically significant improvement in depressive symptoms did not differ between treatment and placebo groups. Although the improvement was small, and the clinical importance was uncertain, vitamin D supplementation and maintaining vitamin D sufficiency could reduce depressive symptoms in patients with knee OA.

Despite the noticeable prevalence of depression in OA patients and the association between vitamin D deficiency and depression, no study has explored the effect of vitamin D supplementation on depression in OA patients. In addition, previous studies that assessed

Table 1
Baseline Characteristics of Participants Between Vitamin D Supplementation and Placebo Groups

Characteristic	Vitamin D Group (n = 209)	Placebo Group (n = 204)
Age, y, mean (SD)	63.5 (6.9)	62.9 (7.2)
Female	106 (52.7)	102 (50.0)
Body mass index, mean (SD)	29.6 (5.4)	29.6 (4.6)
Normal weight	42 (20.1)	23 (11.3)
Overweight	88 (42.1)	90 (44.1)
Obese	79 (37.8)	91 (44.6)
Serum 25(OH)D levels, nmol/L, mean (SD)	43.7 (11.8)	43.8 (12.7)
Regular smokers	92 (44.7)	98 (48.5)
Self-reported depression	10 (4.8)	14 (6.9)
Antidepressant medicine use	6 (3.0)	11 (5.6)
Physical activity		
Insufficiently active	39 (22.2)	37 (20.9)
Sufficiently active	111 (63.1)	114 (64.4)
Highly active	26 (14.8)	26 (14.7)
Highest education		
<High school	26 (12.9)	25 (12.1)
High school	73 (36.1)	78 (37.7)
>High school	103 (51.0)	104 (50.2)
WOMAC score, mean (SD)		
Pain (0–500)	137.9 (88.8)	134.7 (83.4)
Function (0–1700)	487.9 (318.1)	467.6 (292.8)
Stiffness (0–200)	61.5 (41.5)	61.7 (40.1)
PHQ-9 score (0–27)	3.4 (4.1)	3.0 (4.0)
Depression status		
No depression	145 (72.1)	151 (77.0)
Mild depression	38 (18.9)	32 (16.3)
Moderate to severe depression	18 (9.0)	13 (6.6)

SD, standard deviation.
Values are n (%) unless otherwise noted. No depression was defined as PHQ-9 scores of <5; mild depression was defined as PHQ-9 scores 5 to 9; moderate to severe depression was defined as PHQ-9 scores of ≥10. Student *t* test or χ^2 test was used for the comparison.

the effect of vitamin D supplementation on depression have not provided a consensus in the general population, obese, or other diseased populations.[18] The inconsistencies among studies could be attributed to different study samples, different cut-offs for defining vitamin D deficiency, variations in the participants' baseline and follow-up vitamin D levels, and different methodologies used to evaluate depression.[19] Some researchers provided an explanation for their null findings, stating that they did not measure 25(OH)D levels throughout the study to examine if supplementation actually changed serum 25(OH)D, which was described as a "biological flaw" and should be considered as a limitation of the study design. Some studies failed to demonstrate whether participants were vitamin D deficient at baseline or whether they achieved sufficiency during the trial.[18] Additional RCTs are needed to address this "biological flaw."

There was a pilot study that evaluated the effect of vitamin D supplementation on depression in patients with chronic widespread musculoskeletal pain and vitamin D deficiency.[31] It demonstrated that 50,000 IU/week oral vitamin D_3 treatment for 3 months resulted in a prominent improvement in depression, which was assessed using the 21-item Beck Depression Inventory. In the current study, we enrolled participants with vitamin D deficiency at baseline, and most participants achieved vitamin D sufficiency after 24 months of treatment with 50,000 IU of monthly vitamin D_3. We found that significantly more participants achieved vitamin D sufficiency at month 3 in the vitamin D supplementation group than in the placebo group,[20] and depressive symptoms were decreased more in the vitamin D supplementation group from month 6. These results suggest that vitamin D supplementation may have an effect on depressive symptoms when serum vitamin D levels reaches optimal levels. Compared with placebo, vitamin D supplementation significantly reduced depressive symptoms (measured using the PHQ-9 score) in patients with knee OA in this study over 24 months. As we hypothesized, when the vitamin D deficiency was corrected, the vitamin D could have the neuroprotective effect on brain. Vitamin D supplementation in daily, weekly, or monthly dosing frequencies achieves equal vitamin D sufficiency.[32] Therefore, participants receiving a daily or weekly use of same cumulative dosage could reach a similar outcome as those receiving a monthly use. However, a monthly dosage can optimize participants' adherence to long-term vitamin D supplementation study as it is more convenient. One weakness of our study was that we included both depressed and nondepressed participants, though the prevalence of depression was high in our study population compared to the general population aged over 65.[3]

In our RCT, 62% of participants in the placebo group reached a sufficient level of serum 25(OH)D at month 24 as reported previously, which was thought in part to result from seasonal variation, changes in lifestyle, and dietary supplementation.[21] We hypothesized that the high proportion of patients achieving sufficient 25(OH)D levels in the placebo group would dilute the beneficial effects of vitamin D supplementation. Therefore, we performed a post hoc analysis to describe whether maintaining sufficient serum vitamin D has a beneficial effect on depression. Indeed, our findings were consistent with our primary analysis, demonstrating that maintaining sufficient serum vitamin D improved depressive symptoms. In addition, we found that the effects of vitamin D supplementation and maintaining serum vitamin D on depression remained largely unchanged after further adjustment for knee pain or function, suggesting that they were independent of improvements in knee symptoms.

There is limited information regarding an MCID for the PHQ-9 scale. Lowe defined a change of 2.59 as the MCID among individuals with major depression, dysthymia, or both[25]; thus, we used this value as the cut-off point to define a clinically important improvement in depression. In those with moderate to severe depression at baseline,

Table 2
Effects of Vitamin D Supplementation Over 24 Months on Change in PHQ-9

	Baseline Mean (SD)	Month 24 Mean (SD)	Change, Mean (95% CI)	Between-Group Difference Change, Mean (95% CI)	*P* Value
Whole sample					
Placebo group (n = 204)	3.0 (4.0)	3.2 (3.8)	0.21 (−0.19, 0.61)	**−0.66 (−1.22, −0.11)**	**.02**
Vitamin D group (n = 209)	3.4 (4.1)	2.9 (3.4)	**−0.45 (−0.84, −0.07)**		
Those without depression (PHQ-9 <5) at baseline					
Placebo group (N = 151)	1.3 (1.3)	2.0 (2.6)	**0.66 (0.27, 1.04)**	−0.24 (−0.79, 0.30)	.38
Vitamin D group (N = 145)	1.4 (1.3)	1.9 (2.4)	**0.41 (0.03, 0.79)**		
Those with at least mild depression (PHQ-9 ≥5) at baseline					
Placebo group (n = 45)	8.9 (4.5)	6.7 (4.6)	**−1.83 (−3.17, −0.50)**	−0.86 (−2.64, 0.91)	.34
Vitamin D group (n = 56)	8.5 (4.4)	5.4 (4.3)	**−2.70 (−3.86, −1.53)**		

Changes in outcomes are generated from mixed-effect models adjusted for age, sex, and BMI. Between-group differences were calculated by subtracting the vitamin D group values from the placebo group values. Significant values are in bold.

Table 3
Effects of Vitamin D Status Over 24 Months on Change in PHQ-9

	Baseline Mean (SD)	Month 24 Mean (SD)	Change, Mean (95% CI)	Between-Group Difference Change, Mean (95% CI)	*P* Value
Whole sample					
Not maintaining sufficient vitamin D (n = 114)	2.4 (2.9)	2.9 (3.5)	0.36 (−0.20, 0.92)	**−0.77 (−1.45, −0.08)**	**.03**
Maintaining sufficient vitamin D (n = 226)	3.3 (4.1)	2.8 (3.6)	**−0.41 (−0.80, −0.02)**		
Those without depression (PHQ-9 <5) at baseline					
Not maintaining sufficient vitamin D (n = 89)	1.4 (1.4)	2.0 (2.6)	**0.60 (0.12, 1.08)**	−0.15 (−0.75, 0.45)	.62
Maintaining sufficient vitamin D (n = 161)	1.3 (1.3)	1.8 (2.4)	**0.45 (0.09, 0.80)**		
Those with at least mild (PHQ-9 ≥5) depression at baseline					
Not maintaining sufficient vitamin D (n = 18)	7.6 (2.9)	6.9 (3.9)	−0.76 (−2.70, 1.19)	−1.92 (4.14, 0.29)	.09
Maintaining sufficient vitamin D (n = 61)	8.6 (4.5)	5.9 (4.6)	**−2.68 (−3.74, −1.63)**		

Changes in outcomes are generated from mixed-effect models adjusted for age, sex, body mass index, serum 25(OH)D level, PHQ-9 score, self-reported depression, and use of antidepressant medication at baseline. Between-group differences were calculated by subtracting the maintaining sufficient vitamin D group values from the not maintaining sufficient group values.

we did not see any difference in the rate of improvement by treatment groups. However, it should be noted that the prevalence of mild and moderate to severe depression in this current study was 17.6% and 7.8%, respectively, which was considerably lower than those in OA populations from previous studies,[33,34] indicating that a few participants were clinically depressed or had major depression. Because of a lack of MCID for those with milder depressive symptoms, it remains unclear whether the statistically significant improvement in depressive symptoms we reported in this study is clinically important. Notably, the effect size was doubled in those with any depression at baseline, suggesting that the potential therapeutic effect of vitamin D would be most evident in knee OA patients having depression.

This current study had some potential limitations. It was a secondary analysis of an RCT that was primarily designed to examine vitamin D supplementation on knee OA outcomes.[20] Nevertheless, this study is the first to examine the effect of vitamin D supplementation on depression in OA patients, and the findings from this study are biologically plausible. Although the effects were not significantly different in those with depression at baseline, most likely due to reduced sample size, the sizes were doubled in magnitude. It remains unclear whether the improvement in depressive symptoms we reported is clinically significant. Further RCTs are needed to confirm our findings by selecting patients with both knee OA and depression. There may exist unanticipated selection bias, as baseline PHQ-9 score was higher in the group with maintaining vitamin D sufficiency. However, the associations remained significant after adjustments for baseline PHQ-9 score, suggesting the selection bias may not be a concern. In addition, 62% of patients in the placebo group reached sufficient vitamin D levels after 24 months,[21] which might have been due to seasonal change, physical activity, or other reasons. However, we used serum vitamin D levels to detect the association between variation in vitamin D status and depressive symptoms and obtained consistent findings. Furthermore, although the magnitude of the observed changes was small, it is consistent in both parts with the RCT and observational study, suggesting the findings would be real.

Conclusions

Vitamin D supplementation and maintaining sufficient vitamin D levels over 24 months may have beneficial effects on depressive symptoms in patients with knee OA.

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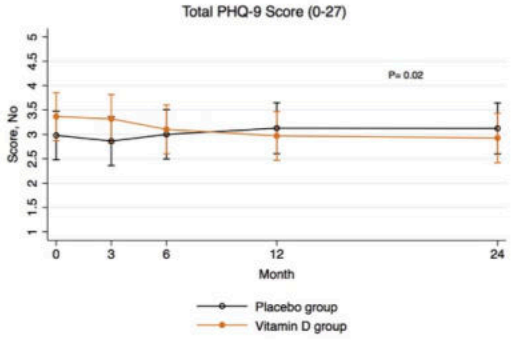

Fig. 2. Change in PHQ-9 scores in the vitamin D supplementation group and the placebo group. Vertical bars indicate 95% CIs for the mean scores. *P* value was for the difference between the 2 groups in PHQ-9 score changes from baseline to month 24.

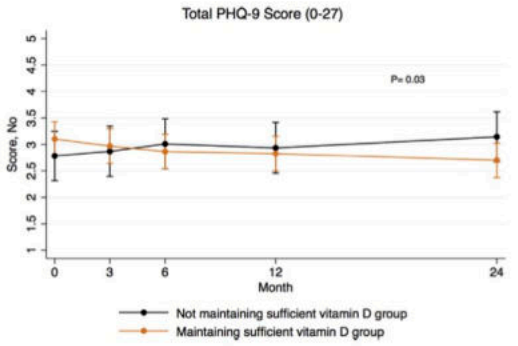

Fig. 3. Change in PHQ-9 scores in the group that maintained vitamin D sufficiency between month 3 and 24 and the group which did not maintain vitamin D sufficiency between month 3 and 24 (Vertical bars indicate 95% CIs for the mean scores. *P* value was for the difference between the 2 groups in PHQ-9 score changes from baseline to month 24).

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